



IntelliPlex™ SARS-CoV-2 Detection Kit

Instructions for Use

REF 82303-U **96 Reactions**

IVD **In Vitro Diagnostic Use**

For use under an Emergency Use Authorization (EUA) only.

For Prescription Use only



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**IMPORTANT: Read the instructions
carefully prior to Use**

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1. INTENDED USE

The *IntelliPlex™ SARS-CoV-2 Detection Kit* is a molecular test based on reverse transcription-polymerase chain reaction (RT-PCR) in combination with π Code technology and the IntelliPlex 1000 π Code Processor and PlexBio 100 Fluorescent Analyzer with DeXipher software, and is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The *IntelliPlex™ SARS-CoV-2 Detection Kit* is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of PCR, PlexBio's instrument platform, and in vitro diagnostic procedures. The IntelliPlex SARS-CoV-2 Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. SUMMARY AND EXPLANATION

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-CoV-2), which has resulted in thousands of confirmed human infections in many countries including the United States. Cases of

asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

The *IntelliPlex™ SARS-CoV-2 Detection Kit* is a molecular *in vitro* diagnostic test that aids in the detection of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers, labeled oligonucleotide probes, and control material used for the *in vitro* qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

3. PRINCIPLES AND PROCEDURE

Coronaviruses are a large family of viruses that may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Severe Acute Respiratory Syndrome (SARS) and Coronavirus Disease 2019 (COVID-19). The COVID-19 is the infectious disease caused by the most recently discovered coronavirus, SARS-CoV-2. This new virus and disease were unknown before the outbreak began in December 2019. In a few months' time, COVID-19 has become a global pandemic, resulting in over four million cases world-wide. SARS-CoV-2 is the single-stranded RNA virus. Detection of SARS-CoV-2 nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage specimens are feasible due to the optimal primer and probe design in combination with π Code MicroDisc technology.

The assay includes primers/probe sets designed to detect SARS-CoV-2 specific target sequences including regions within the RdRP, E, and N genes.

π Code MicroDisc

π Code MicroDiscs are manufactured to generate more than 85,000 distinct circular image patterns for multiplexing applications. Each π Code has a distinct circular image pattern, which corresponds to a specific capture agent conjugated to the surface of the disc. π Code tagged with different capture agents are pooled, enabling specific detection of multiple analytes in one-well reaction.

Detection Principle

The procedure is based on the following processes:

- I. Viral RNA purified from acceptable respiratory specimens using the QIAamp Viral RNA Mini Kit.
- II. RT-PCR amplification of viral RNA.

- III. Hybridization of PCR amplicons with virus-specific probes conjugated to π Code MicroDiscs in a single well reaction.
- IV. Incubation with SA-PE (Streptavidin-phycoerythrin) for fluorescent labeling.
- V. Image pattern decoding and fluorescent signal detection by the PlexBio™ 100 Fluorescent Analyzer.

4. MATERIALS PROVIDED

The *IntelliPlex™ SARS-CoV-2 Detection Kit* contains sufficient reagents for 96 tests. The kit components supplied are listed as follows.

- 1) SARS-CoV-2 KIT Primer Mix**
Ref. No.: 20559-U
Quantity & Volume: 1 vial, 384 μ L
Description: For RT-PCR amplification
Contents: ~4 μ M Primer (including biotin-labeled primers)
- 2) SARS-CoV-2 KIT RT-PCR Buffer**
Ref. No.: 20561-U
Quantity & Volume: 2 vials, 1 mL/vial
Storage: Store at -15°C to -25°C upon arrival
Description: For RT-PCR amplification
Contents: Buffered solution containing MgSO₄ and dNTP
- 3) SARS-CoV-2 KIT RT-PCR Enzyme Mix**
Ref. No.: 20560-U
Quantity & Volume: 1 vial, 96 μ L
Storage: Store at -15°C to -25°C upon arrival
Description: For RT-PCR amplification
Contents: RT/Hot-Start Taq Mix (0.1 to 0.5 Units/ μ L): Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase and HotStart Taq DNA Polymerase, RNase Inhibitor
- 4) SARS-CoV-2 KIT π Code MicroDisc**
Ref. No.: 20562-U
Quantity & Volume: 2 vials, 1 mL/vial
Description: For RT-PCR amplicon capture
Contents: π Code MicroDiscs (8-plex including RdRP, E, N, MS2, GUSB, Blank, SA-PE, Lot ID), Glycerol, Phosphate buffered saline, 0.1% Albumin-from bovine (Biological), <0.1% EDTA and <0.1% Sodium azide
- 5) SARS-CoV-2 KIT POS (Positive) Control**
Ref. No.: 20563-U
Quantity & Volume: 3 vials, lyophilized
Description: Assay positive control; reconstituted

20 µL ddH₂O per vial prior to use. Single use only.
Contents: RNA representing SARS-CoV-2 E, N and RdRP gene mixed with human total RNA; preserved in RNA stable.

6) SA-PE Solution

Ref. No.: 20320-U

Quantity & Volume: 1 bottle, 10 mL/bottle

Description: Streptavidin-phycoerythrin for fluorescent signal acquisition

Contents: Phosphate buffered saline, 0.5% Streptavidin-phycoerythrin, 1% Albumin- from bovine (Biological), <0.1% Sodium azide

7) Hy Buffer

Ref. No.: 20565-U

Quantity & Volume: 1 bottle, 9.6 mL/bottle

Description: For assay hybridization

Contents: Saline-Sodium Phosphate-EDTA, <0.1% Sodium Azide as preservative

8) 10X Wash Buffer

Ref. No.: 20546-U

Quantity & Volume: 2 bottles, 50 mL/bottle

Description: For πCode washing

Contents: Phosphate buffered saline containing surfactant and preservative

9) NEG (Negative) Control

Ref. No.: 20549-U

Quantity & Volume: 1 vial, 1.5 mL

Description: Assay negative control

Contents: Nuclease-free water

10) SARS-CoV-2 KIT Extraction Control

Ref. No.: 20564-U

Quantity & Volume: 1 vial, 1 mL

Description: Assay external control

Contents: MS2 bacteriophage with RNA sequence serving as extraction control

11) ddH₂O

Ref. No.: 20548-U

Quantity & Volume: 1 vial, 1.5 mL/vial

Description: For reconstitution of SARS-CoV-2 KIT POS Control

Contents: Nuclease-free water

NOTE: POS Control, NEG Control and Hy Buffer refer to positive control, negative control and hybridization buffer, respectively.

The kit contains sufficient reagents for 3 independent test runs (including POS and NEG controls) and for a maximum of 96 tests.

REQUIRED BUT NOT PROVIDED

Required products for compatibility with IntelliPlex kits:

- 96-well plate (PlexBio; Cat. No. 80025 or Greiner Bio-one; Cat. No. 655101)
- IntelliPlex™ 1000 πCode Processor (PlexBio; Cat. No. 80033)
- PlexBio 100 Fluorescent Analyzer (PlexBio; Cat. No. 80000)
- IntelliPlex™ Calibration Kit (PlexBio; Cat. No. 80035)
- U Tray (PlexBio; Cat. No. 80023)
- V Tray (PlexBio; Cat. No. 80024)
- DeXipher™ MD (PlexBio; Cat. No. 80051)
 - Lot ID, Completeness πCode MicroDiscs

Required components:

- QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52904/ 52906) or IntelliPrep Nucleic Acid Extraction Kit (PlexBio; Cat. No. 83003) + IntelliPrep Automated Nucleic Acid Extraction System (PlexBio; Cat. No. 80040; software version V2.1)
- Clean tubes for PCR reaction (Gunster; Cat. No. MB-P08A or equivalent)
- Disposable gloves, powder-less
- Dedicated micropipette*
- Filter tips for micropipette*
- ddH₂O for dilution of 10X Wash Buffer
- Vortex mixer
- Micro-centrifuge
- Eppendorf® PCR Cooler or comparable (Recommended)
- Thermocycler: MiniAmp Thermal cycler (Thermo Fisher; Cat. No. A37834; software version 1.1.3)
- Industrial Computer (Recommended: PlexBio; Cat. No. 80002)

* Use dedicated pipettes for sample purification, sample preparation, and sample hybridization. Do not share equipment between procedures. Pipettes should be accurate within 3% of the stated volume. Aerosol barrier or positive displacement DNA- and RNase-free tips must be used

6. WARNINGS AND PRECAUTIONS

- Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For Emergency Use Authorization Only.
- For *In Vitro* Diagnostic Use Only (IVD).
- For Prescription Use Only (Rx).
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA

5. MATERIALS AND EQUIPMENT

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For *In-Vitro* Diagnostic Use/For Use Under an Emergency Use Authorization (EUA) Only/For Rx Use Only

- under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
 - The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner..
 - Positive results are indicative of the presence of SARS-CoV-2 RNA.
 - Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
 - Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
 - Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
 - Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV <https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
 - Please read the package insert carefully prior to operation. The *IntelliPlex™ SARS-CoV-2 Detection Kit* is only for emergency use with a prescription, as an *in vitro* diagnostic test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
 - False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

- Separate, dedicated rooms and equipment for pre- and post-PCR process with a unidirectional workflow to avoid any contaminations is required.
- All pre-PCR steps should be carried out in the laminar flow hood to further reduce contamination risk.
- Do not use a kit or reagent past its expiration date.
- Sample preparation, RT-PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as those published by Clinical And Laboratory Standards Institute; clean all equipment and surface areas regularly (e.g., The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution).
- Component reagents have been diluted optimally. Further dilution of the component reagents is not recommended.
- All chemicals, biological materials and human origin samples should be considered as potentially hazardous and/or infectious and should be treated accordingly.
- Some reagent contains EDTA and/or Sodium Azide in highly diluted concentration. Follow Good Laboratory Practices and Universal Precautions guidelines to avoid any risk.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens, and waste according to applicable central/federal, state, and local regulations.
- Wear powderless gloves and do not touch and make any markings on the bottom of the plate at any time, as fingerprints and markings would interfere with decoding and signal acquisition. Do not mark the top of the plate.
- General laboratory precautions should be taken:
 - Do not pipette by mouth.
 - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
 - Wash hands thoroughly after handling samples and reagents.
- Avoid RNase contamination:
 - Create an RNase-free working environment.
 - Wear gloves during all steps of the procedure.
 - Change gloves frequently.
 - Use only certified RNase-free sterile, disposable polypropylene tubes and filter strips.
 - Keep tubes closed whenever possible during the

- preparation.
- Use RNase removing product to clean bench surfaces, pipettes and other components used in the experiment.
 - Material Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

7. REAGENT STORAGE, HANDLING AND STABILITY

Storage

The RT-PCR Buffer and RT-PCR Enzyme Mix of the *IntelliPlex™ SARS-CoV-2 Detection Kit* should be stored at -15°C to -25°C separately upon arrival.

Other kit components of the *IntelliPlex™ SARS-CoV-2 Detection Kit* should be stored at 2°C to 8°C.

Stability

Do not use the *IntelliPlex™ SARS-CoV-2 Detection Kit* when it is expired. All components are guaranteed up to the expiration date on the label if handled and stored under the recommended conditions.

Transportation

The shipping temperature for the *IntelliPlex™ SARS-CoV-2 Detection Kit* is at 2-8°C. If the kit package or components are incomplete, please contact PlexBio customer service (service@plexbio.com).

8. QUALITY CONTROL

The *IntelliPlex™ SARS-CoV-2 Detection Kit* contains a series of internal control πCode MicroDiscs that monitor the specimen preparation, RT-PCR amplification, SA-PE incubation procedure, and background noise. These controls must always meet specifications and should have approximately the same intensity in each test well in the same test run. Otherwise, the test is invalid. The external controls (positive control and negative control) monitor the whole testing procedure to prevent false-positive or false-negative results. The test is considered invalid if any of the controls fail to meet the specified value.

Controls		Monitored Condition
Assay Performance Controls	Positive Control	RT-PCR amplification and all downstream procedures
	Negative Control	(Extraction contamination control), Non-specific amplification, cross-contamination
Sample Specific	Reference Gene	Specimen quality, RNA extraction, and all

Controls	Monitored Condition
Controls	Control (GUSB)*
	Extraction Control (MS2)
	Blank πCode MicroDiscs
πCode MicroDisc Controls (monitored in each well-internal references)	Hybridization and washing conditions; fluorescence background
SA-PE πCode MicroDiscs	Fluorescence labeling with SA-PE
Lot ID πCode MicroDiscs	Lot expiration date
Completeness of πCode MicroDiscs	Eight πCode MicroDisc types (with five or more MicroDiscs each) must be detected

*GUSB - Human glucuronidase Beta [GUSB] gene mRNA

9. INSTRUMENT AND SOFTWARE

Instrument

Refer to the instrument user manual for complete operation instructions (Thermo Fisher MiniAmp Thermal cycler, IntelliPlex 1000 πCode Processor and PlexBio 100 Fluorescent Analyzer).

Software Installation

The *IntelliPlex™ SARS-CoV-2 Detection Kit* has a designated Kit App and ENC file. The Kit App contains the πCode target assignments and the ENC file includes the lot number and expiration date. Please make sure you have the Kit App installed and the ENC file imported into DeXipher before your first assay run.

Kit App Installation

1. Log into www.plexbio.com and download the *IntelliPlex™ SARS-CoV-2 Detection Kit* App.
2. Click on the “Installer” in the APP folder and follow the instructions to complete the Kit App installation.

NOTE:

The Kit App only needs to be installed once. Version updates will be notified by customer service.

ENC File Installation

1. Log into www.plexbio.com and download the *IntelliPlex™ SARS-CoV-2 Detection Kit* ENC file. Each kit lot number will have a unique ENC file, so you will need to download a new ENC file each time you purchase a kit with a different lot number. Make

- sure to select the ENC file with the lot number that corresponds to your kit.
2. Save the ENC file to your computer.
 3. Follow the PlexBio 100 Fluorescent Analyzer User Manual to import the ENC file.

10. SPECIMENS

This kit is intended to be used with nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal wash/aspirate or nasal aspirates, and bronchoalveolar lavage specimens. Specimen collection, shipping and handling must follow the published guidelines from the Center of Disease Control and Prevention (CDC):

<https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>

Specimen Collection

Follow specimen collection devices manufacturer instructions for proper collection methods.

Swab: Only synthetic fiber swabs with plastic shafts should be used. Do not use calcium alginate swabs or swabs with wooden shafts (inactivate some viruses and inhibit PCR testing). Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

Wash/Aspirate: Use 1 mL-1.5 mL of non-bacteriostatic saline (pH 7.0). Place specimen in a sterile viral transport media tube.

BAL: Samples should be collected into a sterile, leakproof collection cup or dry container without preservative matrix.

Specimen Transport

Suspected and confirmed SARS-CoV-2 patient specimens, cultures, or isolates must be packed and shipped according to UN 3373 Biological Substance, Category B, and in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations. Personnel must be trained to pack and ship according to the regulations and in a manner that corresponds to their function-specific responsibilities.

Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at -70°C or lower, ship overnight on dry ice.

Specimen Storage

If samples cannot be processed immediately upon receipt in the laboratory, specimens can be stored at 2-8°C for up

to 72 hours after collection. If longer storage is expected due to a delay in processing or shipping, store specimens at -70°C or lower.

Purification and Storage of Extracted RNA

Before extraction, add 10 µL SARS-CoV-2 Kit Extraction Control to each specimen. The *IntelliPlex™ SARS-CoV-2 Detection Kit* has been validated using two extraction methods.

QIAamp Viral RNA Mini Kit

Purify samples using QIAamp Viral RNA Mini Kit according to manufacturer's instruction. The minimum specimen volume needed for purification processing is 140 µL, eluted in 50 µL buffer.

IntelliPrep Nucleic Acid Extraction Kit

Purify samples using IntelliPrep Nucleic Acid Extraction Kit according to manufacturer's instruction. Sample extraction requires using the IntelliPrep Automated Nucleic Acid Extraction System. The specimen volume needed for purification processing is 400 µL, eluted in 50 µL buffer.

The NEG Control is included in the extraction process to monitor for contamination and is carried throughout the complete workflow including RT-PCR. A volume of 140 µL NEG Control is required for purification using the QIAamp Viral RNA Mini Kit. A volume of 400 µL NEG Control is required for purification using IntelliPrep Nucleic Acid Extraction Kit. **Extraction Control (MS2) must not be added to the NEG Control.** Elute in 50 µL buffer.

Extracted RNA can be stored at 2°C to 8°C for up to 4 hours, or at -15°C to -25°C for up to 7 days. Long term storage is not recommended.

11. ASSAY PROCEDURE

Warning: Read the instructions carefully and follow every step of the assay protocol correctly.

Important Handling Instructions:

Separate, dedicated areas and equipment for sample purification, sample preparation and sample hybridization must be used. Equipment (including lab coats) must not be shared between areas. All equipment and surface areas should be cleaned before and after each run (e.g., using a 0.5 – 1 % Sodium hypochlorite solution). All work should be performed according to approved guidelines such as those published by Clinical and Laboratory Standards Institute.

11.1 RT-PCR Amplification

1. If stored below -20°C, thaw purified samples on ice (4°C).
2. Label RT-PCR tubes with unique numbers/names assigned. Include one tube for Positive Control and one tube for Negative Control.

Procedure	Wash Buffer Consumption (mL)
Self-test	50
DNA/RNA program (1 lane, up to 8 tests)	150
DNA/RNA program (12 lanes, up to 96 tests)	535

Positive Control must be reconstituted with 20 µL ddH₂O before use. Positive Control vials are single use only. Discard of unused leftovers.

3. Prepare the PCR reaction.

For each PCR reaction:

SARS-CoV-2 KIT RT-PCR Enzyme Mix	1 µL
SARS-CoV-2 KIT RT-PCR Buffer	20 µL
SARS-CoV-2 KIT Primer Mix	4 µL
Sample/PC/NC	15 µL
Total volume	40 µL

NOTE:

- The amount of RT-PCR reaction mix and primer mix required for a Master Mix depends on the number of reactions. Always prepare a surplus.
 - Both POS Control and NEG Control are required for test validity and report generation and must be included in each assay run.
4. Mix by tapping the tubes and spin down before placing the tubes on the thermocycler. Set up the PCR program conditions as shown below:

Step	Temp.	Time	Cycles
RT	55°C	15 min	1
Initial Denaturation	95°C	2 min	1
Denaturation	95°C	15 sec	36
Annealing/extension	60°C	30 sec	
	4°C	∞	1

NOTE: Ramp rate: 3 °C/sec (ABIMiniAmp)

11.2 DNA Hybridization and SA-PE Reaction

- 1) **Prepare 1X Wash Buffer:** Transfer 50mL of the 10X Wash Buffer to the IntelliPlex 1000 πCode Processor 1L Wash Buffer bottle and add 450 mL ddH₂O. Mix by swirling.

NOTE: The prepared 1X Wash Buffer can be used for up to one week.

IntelliPlex 1000 πCode Processor Wash Buffer consumption:

- 2) **Add 20 µL πCode MicroDisc to 96-well plate:** Mix by vortexing the SARS-CoV-2 KIT πCode for 10 seconds, then add 20 µL of the πCode to each well directly. Vortex the tube of πCode every four wells in between dispensing to ensure homogeneous suspension.

NOTE: Each amplified PCR product (including samples, POS and NEG control) should be added into wells respectively in order of A1, B1...H1 and followed by A2, B2...H2 and so on.

- 3) **Dispense 100 µL of Hy Buffer** to each well.
- 4) Spin down the RT-PCR products.
- 5) **Denature the RT-PCR products** on the thermocycler by heating at 95°C for 7 minutes followed by immediate cooling at 4°C (Ramp rate: 100%) immediately.

NOTE: Pay attention to the lid temperature of thermocycler while taking out the denatured PCR products.

- 6) Spin down the RT-PCR products and keep PCR products on ice (4°C; e.g., in Thermocycler or use Eppendorf® PCR Cooler or comparable). Use immediately (within 1 hour after denaturation).
- 7) **Add 20 µL of each freshly denatured sample** to corresponding well of 96-well plate (containing Hybridization buffer and πCode MicroDisc).
- 8) **Add 20 µL freshly denatured Positive Control** sample to corresponding well.
- 9) **Add 20 µL freshly denatured Negative Control** sample to corresponding well.
- 10) **Pipet the required volume of SA-PE solution** into the SA-PE solution tank (V Tray).

Required SA-PE Solution by Lane(s):

Number of Processed Lane(s)	Required SA-PE Solution (µL)
1	900

Number of Processed Lane(s)	Required SA-PE Solution (μ L)
2	1300
3	1700
4	2100
5	2500
6	2900
7	3600
8	4000
9	4400
10	4800
11	5200
12	5600

- 11) **Run hybridization and wash:** This assay uses the **DNA/RNA program** in the **Molecular Assay** window of the IntelliPlex 1000 π Code Processor. Refer to the IntelliPlex 1000 π Code Processor operation manual and follow the instructions to run the built-in assay program (Homepage/ Molecular Assay/ Well Selection/ DNA/RNA/ Confirm procedure conditions / Start Running). The plate will be ready for decoding once the program finished (~60 minutes).

NOTE:

- SA-PE solution should be kept in the dark.
- **Do not** reuse the leftover SA-PE solution and V Tray. Replace a new V Tray with every assay run.
- **Do not** open the door when the instrument is in operation.
- The kit contains sufficient reagents for 3 independent test runs (including POS and NEG controls) and for a maximum of 96 tests. Please note that the included Wash Buffer is only sufficient for up to three independent runs. Additional Wash buffer can be ordered from PlexBio (Ref. No: 80210).

11.3 Image Decoding and Fluorescent Detection

1. Follow the PlexBio 100 Fluorescent Analyzer User Manual to set up the analysis.

NOTE:

- PlexBio 100 Fluorescent Analyzer must be calibrated regularly (once per month) using the IntelliPlex™ Calibration Kit.
- Check that the correct ENC file has been imported.

2. Launch DeXipher to run the qualitative assay.
3. Mark the wells for sample, positive and negative controls.
4. Enter sample information and assay name. Place the plate into the device with the correct orientation as shown on the screen.
5. The raw data will be analyzed through the kit ENC to generate the genotype call report.

NOTE:

- A single run can include from 2 to 96 tests (including POS and NEG controls) per 96 microwell plate.

Step (for 94 specimens)	Time requirements	Description
Sample Extraction	60 minutes	QIAamp Viral RNA Mini Kit or IntelliPrep Nucleic Acid Extraction Kit/IntelliPrep Automated Extraction System
Setup/ Run RT-PCR	90 minutes	MiniAmp Thermal cycler
Hybridization/ SAPE	60 minutes	IntelliPlex™ 1000 π Code Processor
Analyzing Results	60 minutes	PlexBio 100 Fluorescent Analyzer + DeXipher software

12. INTERPRETATION OF RESULTS

The DeXipher software will analyze specimen samples only if the external controls (Positive Control and Negative Control) and internal controls (Reference Gene Control, Blank and SAPE Monitor Control) are all shown as “Pass”. Failed Positive or Negative Control renders the whole assay invalid. Failed Reference Gene Control, Blank or SAPE Monitor Control renders the affected sample invalid.

Please also refer to the chapter “Disclaimer and Limitations” and “Troubleshooting” for additional information.

The RdRP, E, and N targets are all specific to SARS-CoV-2. Detection of any one, two, or three targets is

considered a valid positive result. Only qualitative results of SARS-CoV-2 Detected/Not Detected is shown on the test report.

Result Interpretation

Reported Result	Interpretation	Action
Detected	SARS-CoV-2 positive	Report results to health care provider and appropriate public health authorities.
Not Detected	SARS-CoV-2 negative	
No result reported	Assay was not valid due to external or internal control failure	Troubleshoot to address the control issue or contact PlexBio customer service team; retest or obtain new specimen as necessary.

13. DISCLAIMERS AND LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
- The performance of this test was evaluated using the procedures provided in this product insert. Alteration from the procedure may affect test performance.
- The performance of *IntelliPlex™ SARS-CoV-2 Detection Kit* was established using nasopharyngeal swab samples. Anterior nasal, oropharyngeal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage are also considered acceptable specimen types for use with the *IntelliPlex™ SARS-CoV-2 Detection Kit* but performance has not been established.
- A negative test result means the *IntelliPlex™ SARS-CoV-2 Detection Kit* was unable to detect the virus in the sample. It does not preclude the possibility that the specimen did in fact contain the virus. Only samples with detectable amounts of the virus matching the reference sequences are detected; false negative test results may be due to experimental errors or other causes. Interpretation of the results should consider these possibilities.
- A positive test result means that the *IntelliPlex™ SARS-CoV-2 Detection Kit* was able to determine SARS-CoV-2 in the sample. False positive test results may be caused by experimental errors or other causes. Interpretation of the results should consider these possibilities.

- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the RdRP, E, and N primer/probe sets of the *IntelliPlex™ SARS-CoV-2 Detection Kit*. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- Laboratories are required to report all results to the appropriate public health authorities.

14. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The *IntelliPlex™ SARS-CoV-2 Detection Kit* Letter of Authorization, User Manual, and Labeling are available on the FDA website:

<https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas>

However, to assist clinical laboratories using the *IntelliPlex™ SARS-CoV-2 Detection Kit*, the relevant Conditions of Authorization are listed below:

- Authorized laboratories¹ using *IntelliPlex™ SARS-CoV-2 Detection Kit* will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using *IntelliPlex™ SARS-CoV-2 Detection Kit* will use your product as outlined in the Instructions for Use only. Deviations from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use *IntelliPlex™ SARS-CoV-2 Detection Kit* are not permitted.
- Authorized laboratories that receive *IntelliPlex™ SARS-CoV-2 Detection Kit* will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using *IntelliPlex™ SARS-CoV-2 Detection Kit* will have a process in place for reporting test results to healthcare providers and relevant public health authorities.
- Authorized laboratories will collect information on the performance of *IntelliPlex™ SARS-CoV-2 Detection Kit* and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUAResporting@fda.hhs.gov) and PlexBio Co. Ltd. (Adverse event reporting:

<https://www.plexbio.com/intelliplex%2084%A2-sars-cov-2-detection-kit>) if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product.

- F. All laboratory personnel using *IntelliPlex™ SARS-CoV-2 Detection Kit* must be appropriately trained in molecular techniques and use appropriate laboratory and personal protective equipment when handling this kit and use *IntelliPlex™ SARS-CoV-2 Detection Kit* in accordance with the authorized labeling.
- G. PlexBio Co. Ltd., authorized distributors, and authorized laboratories using *IntelliPlex™ SARS-CoV-2 Detection Kit* will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

¹ The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”

15. ANALYTICAL PERFORMANCE

Limit of Detection (Analytical Sensitivity)

QIAamp Viral RNA Mini Kit

Limit of detection (LoD) was determined as the lowest concentration of SARS-CoV-2 that can be detected at a ≥95% positive rate with the *IntelliPlex™ SARS-CoV-2 Detection Kit*. The LoD was estimated by performing a titration of SeraCare AccuPlex SARS-CoV-2 RNA material (Cat # 0505-0126) in nasopharyngeal swab matrix and confirmed by testing an additional 20 replicates at the estimated LoD target concentration. The confirmed LoD was 140 copies/mL in nasopharyngeal swab matrix.

Replicate	Median Fluorescence Intensity (MFI)*			Detection of SARS-CoV-2
	RdRP	E	N	
140 copies/mL				
Average MFI	11883	8693	13003	20/20 (100%)
70 copies/mL				
Average MFI	787	832	2011	16/20 (80%)

*MFI – values after subtraction of the threshold

IntelliPrep Nucleic Acid Extraction Kit

The LoD was estimated by performing a titration of viral SARS-CoV-2 RNA in nasopharyngeal swab matrix and confirmed by testing an additional 20 replicates at the estimated LoD target concentration prepared using SARS-CoV-2 RNA derived from a natural clinical sample (Access Biologics; ID 2005021504). The confirmed LoD for nasopharyngeal swab matrix was 100 copies/mL.

Because the LoD with the IntelliPlex Nucleic Acid Extraction Kit was determined with a different source of SARS-CoV-2 RNA than that used to establish the LoD with the QIAamp Viral RNA Mini Kit, a bridging study was conducted using whole viral genomic SARS-CoV-2 RNA (Access Biologicals; ID 2005021504) to demonstrate that the LoD is similar when nucleic acid extraction is performed by either method. Whole viral SARS-CoV-2 RNA was spiked into pooled negative clinical nasopharyngeal swab matrix at concentrations of 140 and 70 copies/mL and tested following extraction using the QIAamp Viral RNA Mini Kit (20 replicates per concentration). The results of the study are summarized in the table below.

SARS-CoV-2 Concentration	Detected	Not Detected	Total	Agreement
140 copies/mL	19	1	20	95.0%
70 copies/mL	10	10	20	50.0%

The *IntelliPlex™ SARS-CoV-2 Detection Kit* generated comparable LoD results irrespective of the method of nucleic acid extraction.

Inclusivity (Analytical Reactivity)

BLASTn analysis query alignments were performed with the SARS-CoV-2 E, N and RdRP oligonucleotide primer and probe sequences with full length or near-full-length (>29 kb) nucleic acid sequences for SARS-CoV-2 in NCBI’s Severe Acute Respiratory Syndrome Coronavirus 2 Data Hub (<https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/>). The analysis demonstrated the following:

- 1 mismatch in the “N F Primer” binding site (GenBank: MT246456, MT263410, MT291836 and MT293159)
- 2 mismatches in the “N R Primer” binding site (GenBank: MT263411)

- 3 mismatches in the “N R Primer” (GenBank: MT258379, MT259250, MT259263, MT246470, MT246451, MT233522)
- 1 mismatch in the “E probe” (GenBank: MT263433).

The primers and probe set targeting the RdRP had no mismatch against any of the published sequences.

Despite these mutations in SARS-CoV-2, the IntelliPlex assay is still expected to detect all SARS-CoV-2 strains. Because the assay detects 3 targets that are specific to SARS-CoV-2, even if new or not previously reported nucleotide mutations affect amplification/detection of one of the targets, the presence of the other two targets can still generate a valid positive result.

Cross-Reactivity (Analytical Specificity)

Cross-reactivity of the *IntelliPlex™ SARS-CoV-2 Detection Kit* was evaluated by *in silico* analysis and by performing wet lab testing.

BLASTn analysis queries of the *IntelliPlex™ SARS-CoV-2 Detection Kit* primers and probes were performed against the sequences of the organisms listed in the table below.

The *in silico* analysis predicted that SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross react with the RdRP, E, and N primer/probe sets of the IntelliPlex SARS-CoV-2 Detection Kit.

The RdRP F primer showed homology > 80% to human coronavirus HKU1 (90.9%) and OC43 (90.9%). The RdRP R primer showed homology > 80% to human coronavirus HKU1 (82.7%), OC43 (82.7%), NL63 (89.6%) and MERS coronavirus (82.7%). The RdRP probe showed homology >80% to HKU1 (81.2%), OC43 (81.2%) and MERS coronavirus (87.5%).

The reverse primer for the Reference Gene Control (GUSB R Primer) showed homology > 80% to *Pseudomonas aeruginosa* sequences, and the forward primer for SARS-CoV-2 E gene showed homology > 80% to *Pseudomonas aeruginosa* sequences. None of the other amplification primers showed homology > 80% to any of the sequences included in the analysis.

The RdRP probe sequence showed high homology (>80%) to off-target sequences. Specifically, the probe is 100% complementary to *Candida albicans* and shows homology >80% to *Mycoplasma pneumoniae*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. In addition, the GUSB probe sequence showed high homology (>80%) to off-target sequences.

The probe is >80% complementary to *Candida albicans* and *Pseudomonas aeruginosa*, and *Pneumocystis jirovecii*.

High priority pathogens from the same genetic family		
Organism	Taxid	Notes
SARS coronavirus	694009	100% homology to RdRP F Primer; 96.5% homology to RdRPR Primer; 93.8% homology to RdRP Probe; 100% homology to EF Primer; 100% homology to ER Primer; 83.3% homology to E Probe; 90.9% homology to NF Primer; 90.9% homology to NR Primer; 80% homology to N Probe;
Human coronavirus 229E	11137	No homology >80%
Human coronavirus OC43	31631	90.9% homology to RdRP F Primer; 82.7% homology to RdRPR Primer; 80.2% homology to RdRP Probe
Human coronavirus HKU1	290028	90.9% homology to RdRP F Primer; 82.7% homology to RdRPR Primer; 80.2% homology to RdRP Probe
Human coronavirus NL63	277944	89.6% homology to RdRP R Primer
MERS coronavirus	1335626	82.7% homology to RdRP R Prime; 87.5% homology to RdRP Probe

High priority organisms likely in the circulating area		
Organism	Taxid	Notes
Adenovirus 4	28280	No homology >80%
Adenovirus 7	10519	No homology >80%
HMPV	162145	No homology >80%
Human parainfluenza 1 virus	12730	No homology >80%
Human parainfluenza 2 virus	1979160	No homology >80%
Human parainfluenza 3 virus	11216	No homology >80%
Human parainfluenza 4a virus	11224	No homology >80%
Human parainfluenza 4b virus	11226	No homology >80%
Human Influenza A Virus	11320	No homology >80%

High priority organisms likely in the circulating area		
Organism	Taxid	Notes
Influenza B virus	11520	No homology >80%
Human enterovirus EV68	42789	No homology >80%
Human respiratory syncytial virus	11250	No homology >80%
Rhinovirus	12059	No homology >80%
<i>Chlamydia pneumoniae</i>	83558	No homology >80%
<i>Haemophilus influenzae</i>	727	No homology >80%
<i>Legionella pneumophila</i>	446	No homology >80%
<i>Mycobacterium tuberculosis complex</i>	77643	81.3% homology to MS2 probe
<i>Streptococcus pneumoniae</i>	1313	No homology >80%
<i>Streptococcus pyogenes</i>	1314	No homology >80%
<i>Bordetella pertussis</i>	520	No homology >80%
<i>Mycoplasma pneumoniae</i>	2104	80% homology to RdRP probe
<i>Pneumocystis jirovecii</i>	42068	81.3% homology to GUSB probe
<i>Candida albicans</i>	5476	100% homology to RdRP probe; 93.8% homology to GUSB probe
<i>Pseudomonas aeruginosa</i> group	136841	80% homology to RdRP probe; 81% homology to EF primer; 85% homology to GUSB R primer; 81.3% homology to GUSB probe
<i>Staphylococcus epidermidis</i>	1282	93.8% homology to RdRP probe

Cross-reactivity wet testing was performed to demonstrate that the *IntelliPlex™ SARS-CoV-2 Detection Kit* does not react with other organisms that are reasonably likely to be encountered in the clinical specimen. The study included the organisms listed below using the Zeptometrix NATrol Respiratory Panel 2 (Catalog Number: NATRVP2-BIO), NATrol BC/GM Panel (Catalog Number: NATBCGN-NNS), Zeptometrix: NATrol BC/GP Panel and (Catalog Number: NATBC/GP-NNS) and *C. albicans* strain from the Taiwan Bioresource Collection and Research Center (BCRC).

All organisms were tested in triplicate and at high pathogen concentrations (>10⁶ copies/assay). None of the wet-tested organisms produced a positive signal for the *IntelliPlex™ SARS-CoV-2 Detection Kit*.

Organism	Source	Results
Human coronavirus 229E	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human coronavirus OC43	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human coronavirus HKU1	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human coronavirus NL63	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Human Metapneumovirus (hMPV)	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 1	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 2	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 3	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Parainfluenza virus 4	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza A -H1	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza A -H1 2009	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza A -H3	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Influenza B	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Respiratory syncytial virus	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
Rhinovirus	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
<i>Chlamydia pneumoniae</i>	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
<i>Streptococcus pyogenes</i>	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
<i>Bordetella pertussis</i>	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
<i>Pseudomonas aeruginosa</i>	Zeptometrix (NATBCGN-NNS)	No Cross Reactivity
<i>Staphylococcus epidermidis</i>	Zeptometrix (NATBC/GP-NNS)	No Cross Reactivity
<i>Mycoplasma pneumoniae</i>	Zeptometrix (NATRVP2-BIO)	No Cross Reactivity
<i>Candida albicans</i>	BCRC	No Cross Reactivity

16. CLINICAL STUDY

Testing of Contrived Samples

The performance of the *IntelliPlex™ SARS-CoV-2 Detection Kit* was evaluated using contrived clinical nasopharyngeal (NP) swab specimens. Samples were prepared by spiking SARS-CoV-2 Reference Material (SeraCare; AccuPlex SARS-CoV-2 Reference Material

Kit, Cat. No. 0505-0126) at different concentrations into individual, unique confirmed negative nasopharyngeal (NP) swab matrix. Negative NP swab samples were also tested. RNA was extracted using the QIAamp RNA mini kit. Samples were blinded and randomized for testing.

The results are shown below.

Performance Evaluation

Conc. RNA RdRP, E and N Gene	2x LoD (280 copies/mL)	1x LoD (140 copies/mL)	Negative
Number of NP Swabs	10	20	30
Detection Rate	10/10	20/20	0/30
Agreement with Expected Result (%)	100	100	100

Testing of Clinical Patient Samples

A clinical evaluation study of the *IntelliPlex™ SARS-CoV-2 Detection Kit* was conducted with de-identified natural leftover clinical nasopharyngeal swab specimens that were previously collected for SARS-CoV-2 testing. All specimens were evaluated with an FDA authorized comparator assay. A total of 62 patient specimens (30 positive and 32 negative samples for SARS-CoV-2) were included in the study.

All specimens tested with the *IntelliPlex™ SARS-CoV-2 Detection Kit* were blinded and followed the instructions within the IFU. The *IntelliPlex™ SARS-CoV-2 Detection Kit* detected SARS-CoV-2 in 30 of 30 positive specimens and reported 31 of 32 negative specimens.

		FDA Authorized Molecular Comparator Assay ¹		
		Positive	Negative	Total
<i>IntelliPlex™ SARS-CoV-2 Detection Kit</i>	Positive	30	1	31
	Negative	0	31	31
	Total	30	32	62
Positive Percent Agreement		30/30; 100% (88.65%-100%) ²		
Negative Percent Agreement		31/32; 96.88% (84.26%- 96.88%) ²		

¹ Testing of low positive specimens based on the comparator is still pending

² Two-sided 95% score confidence intervals

The Positive Percent Agreement (PPA) for *IntelliPlex SARS-CoV-2 Detection Kit* was 100 % (30/30) and the Negative Percent Agreement (NPA) for *IntelliPlex*

SARS-CoV-2 Detection Kit was 96.88 % (31/32).

FDA SARS-COV-2 REFERENCE PANEL TESTING

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the QIAamp Viral RNA Mini Kit and MiniAmp thermal cycler, respectively. The results are summarized in the Table below.

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross- Reactivity
SARS- CoV-2	Nasopharyngeal Swab	0.6 x 10 ⁴ NDU/mL	N/A
		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

17. TROUBLESHOOTING

- The troubleshooting steps listed below address possible problem causes and solutions that could be experienced during the assay procedures. If the problem persists after completing the recommended steps in the table provided below, please contact PlexBio customer service immediately for further assistance.
- Specimens may be retested using the previously extracted nucleic acid if stored according to requirements. In the event re-extraction of viral RNA is required, access the original, properly stored sample and repeat the full assay procedure. If the repeat testing still results in a failure, it is recommended to obtain a new patient specimen.
- In the event of a Positive Control failure, the sample may be re-tested using the previously extracted nucleic acid and stored according to requirements. In the event of a Negative Control failure due to contamination, it is recommended to re-extract the original properly stored sample and repeat the full

assay procedure. If the Negative Control failed because of πCode MicroDiscs failure, retesting using residual extracted specimen RNA is accepted. If the Reference Gene Control or Extraction Control has failed, extract new nucleic acid before testing. If testing is affected by specimen quality, a new specimen is required. If a πCode MicroDiscs control failed, retesting using residual extracted specimen RNA is accepted.

Problem	Possible Cause	Recommendations
No Valid Assay Assigned	<ol style="list-style-type: none"> 1. No plate inserted. 2. Plate inserted in wrong orientation. 3. No assay APP installed. 4. No ENC file imported. 5. Two or more lots of reagent used. 	<ol style="list-style-type: none"> 1. Confirm plate is inserted and repeat reading. 2. Confirm orientation of plate and repeat reading. 3. Install assay APP and repeat reading. 4. Import ENC file and repeat reading. 5. One reagent lot used at a time.

Problem	Possible Cause	Recommendations
Positive Control Fail	<ol style="list-style-type: none"> 1. No Positive Control added. 2. RNase contamination. 3. Assay did not work. 4. Wrong PC well selected. 5. “πCode MicroDiscs Combination”, “Blank Control”, “πCode MicroDiscs Count”, or “SAPE Monitor Control” failed 	<ol style="list-style-type: none"> 1. Ensure Controls are added. Ensure proper reconstitution of Positive Control as described. Repeat testing using residual extracted sample for RT-PCR. 2. Ensure all operating procedures are followed correctly and work environment is free of RNase. Repeat testing using newly extracted sample. 3. Make sure all the assay procedures are followed correctly. Ensure all components are stored at required storage conditions. Repeat testing using residual extracted sample for RT-PCR. If there is a general problem with assay performance, obtain new assay kit. 4. Choose the correct PC well and repeat reading. 5. See section below for more details. Repeat testing using residual extracted sample for RT-PCR.

Problem	Possible Cause	Recommendations	Problem	Possible Cause	Recommendations
NegativeControlFail	1. Cross-contamination between samples 2. Wrong NC well selected. 3. “πCode MicroDiscs Combination”, “Blank Control”, “Reference Gene Control”, “πCode MicroDiscs Count”, or “SAPEMonitor Control” failed	1. Clean all surfaces and equipment. Instruction in Package Insert on utilizing different rooms and unidirectional workflow must be followed. Repeat testing using newly extracted sample. 2. Choose the correct NC well and repeat reading. 3. See section below for more details. Repeat testing using residual extracted sample for RT-PCR.	SAPE MonitorControlFail	1. No SA-PE was added or insufficient SA-PE solution for dispensing. 2. SA-PE solution deactivated. 3. Incorrect tested lanes of microplate selected for SA-PE solution dispensing.	1. Make sure all the assay procedures are followed correctly. Calculate sufficient SA-PE solution volume for dispensing. Repeat testing using residual extracted sample for RT-PCR. 2. Ensure correct storage condition and minimize the light exposure. Do not use SA-PE past its expiration date. 3. Repeat assay using residual extracted sample for RT-PCR and make sure lanes are selected correctly.
πCodeMicroDiscs Combination Fail	1. πCode MicroDiscs from a different assay/lot are used. 2. Missing πCode MicroDiscs due to wrong operation	1. Use πCode MicroDiscs provided with the <i>IntelliPlex™ SARS-CoV-2 Detection Kit</i> and ensure the lot-ENC is available. 2. Refer to “πCode MicroDiscs Count Fail” below.	BlankControlFail	1. Wrong hybridization conditions. 2. Residues of SA-PE solution in wells after hybridization.	1. Ensure correct hybridization program is selected. Repeat testing using residual extracted sample for RT-PCR. 2. Ensure all buffers (Wash buffer and ddH ₂ O) on IntelliPlex 1000 Processor are fresh-made and sufficient for washing procedures. Repeat testing using residual extracted sample for RT-PCR.
πCodeMicroDiscs Count Fail	1. πCode MicroDiscs are not properly dispersed in the well. 2. Not enough πCode MicroDiscs added to well. 3. Microbes exist in Wash buffers. 4. Instruments error or malfunction.	1. Re-disperse the microplate using IntelliPlex 1000 Processor, and repeat reading. 2. Ensure πCode MicroDiscs are well-mixed with proper amount added. Repeat using residual extracted sample for RT-PCR. 3. Use freshly prepared wash buffer and ddH ₂ O for hybridization to reduce πCode MicroDiscs loss rate. Repeat using residual extracted sample for RT-PCR. 4. Contact PlexBio Customer Service.	3. PlexBio 100 Fluorescent Analyzer is not calibrated. 4. Markings on plates.	3. Perform calibration on PlexBio 100 Fluorescent Analyzer. Repeat testing using residual extracted sample for RT-PCR. 4. Do not make any marking on the plate. Repeat testing using residual extracted sample for RT-PCR.	

Problem	Possible Cause	Recommendations
Extraction Control Fail	<ol style="list-style-type: none"> The Extraction Control was not correctly added to the specimen Problem during nucleic acid purification/extraction. 	<ol style="list-style-type: none"> Follow the instruction provided in the Package Insert. Repeat testing using newly extracted sample. Follow the instruction provided by the manufacturer of the nucleic acid purification kit. Ensure all buffers are freshly prepared. Repeat testing using newly extracted sample.
Reference Gene Control Fail	<ol style="list-style-type: none"> Poor specimen sample quality RNA purification failed or PCR inhibitors existed. PCR procedures are not performed correctly. RNase contamination. Hybridization did not work. 	<ol style="list-style-type: none"> Specimen was not collected, transported, or stored according to requirements. Follow instructions of sample extraction carefully. Ensure required temperature ranges and centrifugation needs are complied. Ensure complete removal of ethanol. Repeat testing using newly extracted sample. Make sure all PCR procedures are followed correctly. Do not use expired materials or mixed lots of reagents. Ensure storage conditions are correct. Repeat testing with residual extracted sample for RT-PCR. Ensure all the operating procedures are followed correctly. Ensure work environment is free of RNase. Repeat testing using newly extracted sample. Make sure all the assay procedures are followed correctly. Ensure samples are freshly heat-denatured. Repeat testing using residual extracted sample for RT-PCR.

18. SYMBOLS

Symbol	Explanation	Symbol	Explanation
	In-vitro diagnostic use		Catalog number
	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
	Contains sufficient for <n> tests		Date of Manufacture
Rx Only	For Prescription Use Only		

19. CONTACT INFORMATION AND PRODUCT SUPPORT

For technical and product support, contact:

service@plexbio.com

Service hotline:

+886-2627-5878

Office hour: 09:00-18:00 (GMT+8)

U.S. Technical and Product Support:

+1 (650) 420-9517

Product support website:

www.plexbio.com

Notice to User

The use of this product and the associated PlexBio instrumentation is covered by one or more issued (US10302640B2, US10436778B2, US10436776B2, US9063044B2, US10019815B2) and pending US and foreign patents owned by PlexBio Co., Ltd. The purchase of this product includes nontransferable rights to use this amount of the product to practice the methods described therein. No general patent or other license of any kind other than this specific right of use from purchase is granted. Further information on purchasing licenses for other applications can be obtained from PlexBio Co., Ltd. 6F-1, No. 351, Yangguang St., Neihu District, Taipei City 11491, Taiwan.

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IntelliPrep Automated Nucleic Acid

Extraction System

(Product Code: 80040)

User Manual

PlexBio Co., Ltd.



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<http://www.plexbio.com>

- For Emergency Use Authorization Only.
- For *In Vitro* Diagnostic Use Only (IVD).
- For Prescription Use Only (Rx).
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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The terms and conditions herein (hereinafter referred to as the “Standard Terms”) apply to all of the products and services provided or will be provided by **PlexBio Co., Ltd.** (hereinafter referred to as “**PlexBio**”). Any changes to the Standard Terms shall have no binding effect on **PlexBio** unless the same has been agreed upon by an authorized representative of **PlexBio** in writing. The “Dealer” referred to herein represents **PlexBio** (if the products are purchased from **PlexBio** directly), or the distributor licensed by **PlexBio**. **PlexBio** expressly disagrees with and hereby rejects any terms or conditions **PlexBio** entered into with the buyer in the purchase order or any of such, which is different from or not included in this Standard Terms, except for agreements made directly between **PlexBio** and the buyer, which unequivocally, explicitly, and expressly states in writing, that **PlexBio** makes certain warranties or quality assurance for COVID-19 related instrument Products authorized under Emergency Use Authorization in 2020 and those warranties or quality assurance for such Product(s)’s terms or conditions shall control, should inconsistency regarding warranties or quality assurance arises between those terms and conditions and this User Manual. In which case, **PlexBio** will honor the certain warranties or quality assurance **PlexBio** makes with buyer in those agreements.

THE BUYER’S ACCEPTANCE, UNPACKING, OR USE OF THE PRODUCTS OR SERVICES SHALL CONSTITUTE HIS/HER ACKNOWLEDGMENT AND ACCEPTANCE OF THE STANDARD TERMS UNCONDITIONALLY. ONCE THE BUYER OPENS THE PACKAGING OF PLEXBIO PRODUCTS OR USES THE PRODUCTS OR SERVICES IN ANY MANNER, THE BUYER ACKNOWLEDGES AND ACCEPTS THE STANDARD TERMS UNCONDITIONALLY, AND ALSO AGREES THAT THE STANDARD TERMS CONSTITUTE THE CONTRACT, WHICH HAS BINDING EFFECT ON THE BUYER UNDER LAW. WHERE THE BUYER DISAGREES WITH THE STANDARD TERMS, HE/SHE SHALL CONTACT PLEXBIO IMMEDIATELY TO RETURN THE PRODUCTS BEFORE USING THE PRODUCTS OR SERVICES IN ANY MANNER, AND HE/SHE SHALL NOT USE THE PRODUCTS OR SERVICES IN ANY MANNER.

PlexBio has the right to amend the terms and conditions regarding specification and service without notifying the buyer in advance if no specification or service has been specified and identified in writing before.

Warranty

The warranty terms and conditions in this Standard Terms (the “Warranty”) apply to any instruments, spare parts, and services (collectively, the “Products”) purchased by the buyer from **PlexBio** directly and situated in the territory of Taiwan. The Warranty excludes any activities other than calibration, certification, and maintenance. **PLEXBIO WILL NOT PROVIDE ANY WARRANTY TOWARD THE SALE AND USE OF THE PRODUCTS OUTSIDE THE TERRITORY OF TAIWAN.** THE PRODUCTS DISTRIBUTED OUTSIDE THE TERRITORY OF TAIWAN WILL BE SOLD “AS IS”. **PlexBio** will provide the buyer with quality assurance toward the spare parts purchased from **PlexBio** and that are used to maintain **PlexBio** instruments under the same terms and conditions herein in any countries/territories other than Taiwan around the world.

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- (i) The buyer shall notify **PlexBio** in writing of the issue of the Products immediately and also provide the details about the issue verified.
- (ii) The buyer shall contact **PlexBio** or the service maintenance engineers trained and qualified by **PlexBio** to evaluate the problems and verify the issues, and buyer might need to bear the related expenses derived from the maintenance or transportation of the Products.
- (iii) Once PlexBio has authorized in prior written consent the buyer to return the Product at issue, the buyer shall bear the cost of freight (including handling and insurance) for any return under quality assurance. PlexBio might inspect and analyze the Products at issue once it has been returned to PlexBio by the buyer.
- (iv) However, if PlexBio verifies issues exists within the returned Products after the said inspection and analysis, PlexBio shall refund buyer the cost of freight shipped from buyer to PlexBio under this authorized quality assurance return. PlexBio will not refund buyer the cost of freight if no issue is found in the returned Products, even if the return has been authorized by PlexBio.
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Chapter 1: Introduction

1.1 Instructions

Before using IntelliPrep Automated Nucleic Acid Extraction System, please read this manual carefully. It provides essential information regarding the IntelliPrep Automated Nucleic Acid Extraction System:

- Safety information
- Symbols
- System overview
- Installation
- Starting extraction
- Maintenance
- Getting help

1.2 Symbols and Regulatory Labels

There are important symbols throughout this manual or on the product with different meanings, including identification, conditions, and safety warnings.

List of Symbols

General cautions and warnings	Caution: pinch point	Warning: Biohazard
	Consult instructions for use	Manufacturer
Date of Manufacture	RUO For Research Use Only	Serial number
Waste Electrical and Electronic Equipment		



The exclamation point within an equilateral triangle is intended to alert the user that the important information of operation and maintenance procedures can be found in the documents with products



Samples may contain biohazardous, infectious materials. Please use personal protective equipment (PPE) including gloves and laboratory coats during operation. Please handle with extreme care when loading the strips onto the stage to prevent spilling.



High temperature warning, do not touch the heating block until it has been cooled.



Waste Electrical and Electronic Equipment

Within the European Union, the Waste Electrical and Electronic Equipment Directive 2002/96/EC requires that you properly dispose of electrical and electronic equipment when it reaches the end of its lifecycle.

Properly dispose of electrical and electronic equipment according to federal, state, and local regulations and requirements.



**IntelliPrep Automated Nucleic Acid
Extraction System**

Model: HF24



Cat. No.: 80040
Input: 100-240V, 50/60 Hz
Power: Max, 400W



YYYY-MM



Manufacturer For:
PlexBio Co., Ltd.
6F-1 No. 351 Yangguang St.,
Neihu Dist., Taipei 11491 Taiwan



XXXXXXXXXXXXXX



RUO

ERP: 80040-R

IntelliPrep Automated Nucleic Acid Extraction System label on the side of the instrument

1.3 Caution and Warning

- 1 **Read the instructions** - before operating the product, you must read all safety and operating instructions.
- 2 **Keep the instructions** - safety and operating instructions should be kept for future reference.
- 3 **Follow the instructions** - all operating and usage instructions must be followed.
- 4 **Cautions and warnings** - you should be aware of all warnings on the product and in the operating instructions.
- 5 **Cleaning** - Unplug the product before cleaning. Only use 75% alcohol to clean the surface of the instrument.
- 6 **Spare Parts** - Do not use any spare parts that are not recommended by the product manufacturer, as they may cause a problem.
- 7 **Liquid and Moisture** - Do not use this product near water - for example, near a laboratory sink to prevent liquid from entering the system. Please contact customer service if liquids accidentally entered the system.
- 8 **Instrument Placement** - Make sure the area is clean, clear, and level. The lab bench must be steady and supporting of the instrument's weight (80kg/ 177lbs). Do not use a rolling table or bench space with overhanging shelves. Do not place this product on an unstable surface such as a cart, shelf, or tripod as it might collapse and cause damage.
- 9 **Ventilation** - The air outlets are designed for ventilation to ensure reliable operation of the instrument, and to protect it from excessive heat. Must not block or cover the air outlets.
- 10 **Power supply** - This product can only be operated with the correct power supply shown on the label. If you are unsure of your electrical source type, please consult your dealer or local power company.
- 11 **Grounding or Polarization** - Use only power cords supplied with the system or cords with grounded outlets properly rated for the system. Ensure the power cord is connected to properly grounded AC outlets only.
- 12 **Power cord protection** - The power cords should be placed carefully so that they cannot be stepped on or squeezed by objects placed on or against them.
- 13 **Lightning** - To protect the product, please unplug the instruments when it is going to be left unused for a longer period. This will prevent possible damage to this product from lightning and power surges. Additionally, it is recommended to use a surge protector.
- 14 **Electric overload** - Do not overload an extension lead or power socket by plugging in appliances together that will exceed the maximum current limit, this could cause fire or electric shock.
- 15 **Items and liquid entry** - Never put any unrelated item inside the instrument, as they may cause a fire, short circuit, or electric shock. Do not spill any kind of liquid on or in the instrument.
- 16 **Defects** - Unplug the product and contact qualified service personnel under the following conditions:

- (1) If the product does not operate according to the instructions. Do not attempt to repair. Improper repair or adjustment may cause a hazardous situation.
 - (2) If the product has been dropped or damaged
 - (3) When the performance of the product is compromised significantly, which indicates that service is needed.
- 17 **Repair** - Do not attempt to repair this product by yourself without the manufacturer's permission and instructions. Opening or removing the cover may expose you to high voltage or other hazards. Unauthorized repair attempts may damage the system. Please contact qualified service personnel for all repairs.
- 18 **Safety inspection** - Authorized service personnel will perform a safety inspection after product service or maintenance to confirm that the product is in normal working condition
- 19 **Heat** - Product should be placed far away from heating sources, such as radiators, open fires (i.e. Bunsen burner), or other products that generate heat.

Chapter 2: IntelliPrep Automated Nucleic Acid Extraction System Overview

2.1 Intended Use

IntelliPrep Automated Nucleic Acid Extraction System is a benchtop instrument, designed to perform fully automated isolation and purification of nucleic acids from blood, tissue, or cell samples. The system is built for use with PlexBio extraction kits, based on silica magnetic bead technology, with a simple bind-wash-elute procedure to eliminate filtration and centrifugation. The system is intended to be used by professional users who are trained in molecular biological techniques. The IntelliPrep Automated Nucleic Acid Extraction System is for research use only, not for use in diagnostic procedures.

2.2 System Specifications

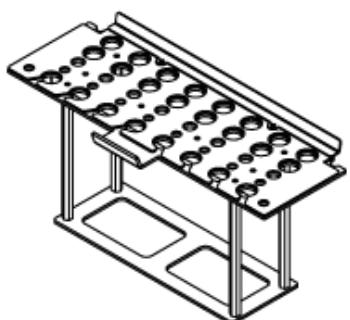
Model	HF24
Throughput	Up to 24 samples per run
System Components	<ol style="list-style-type: none">1. Pipetting Unit: X and Y-axis movement for liquid transfer and dispense.2. Electronic Controller: PLC module and embedded ARM motherboard3. UV Light: Power 8W, stable for 10,000 hours.4. Heating Rod: RT-90°C.5. Display Screen: 7-inch colored touch screen.6. Accessories: Sample Rack, Reagent Strip Rack.
Storage Temperature	18~30 °C / 64~86 °F
Operation Temperature	15~30 °C / 59~86 °F
PERFORMANCE	
Processing Time	60 minutes, varies by kit
Sample Type	Extraction of viral and bacterial nucleic acid from whole blood, FFPE, swab, plasma, or cell suspension
Throughput	1 – 24 samples
Sample Volume	200 – 4000 µL, varies by kit
Elution Volume	30 – 200 µL, varies by kit
Nucleic Acid Purity	DNA: A260/280 = 1.8 ± 0.1 RNA: A260/280 = 1.9 ± 0.1
PHYSICAL CHARACTERISTICS	

Power	100-240V, 50-60Hz
Dimensions	Width: 800 mm / 31.5 inches Depth: 658 mm / 26 inches Height: 669 mm / 26.5 inches
Weight	80 kg (177 lbs)

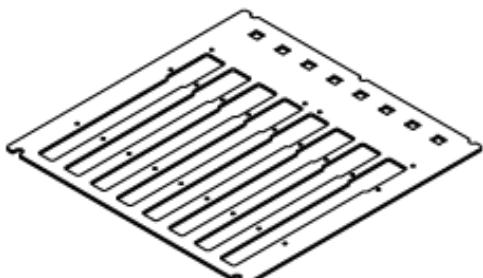
Chapter 3: Installation

3.1 Accessories List

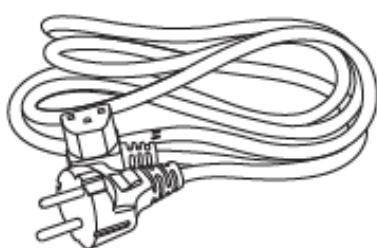
1



2



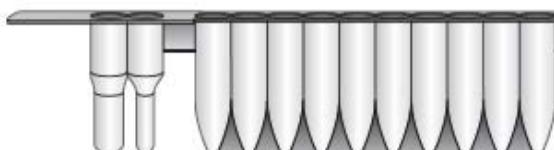
3



4



5



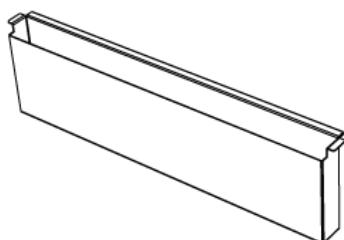
6



7



8



1. Sample Rack x 2

3. Power cord x 1

5. Empty reagent strip x 25

7. Pipette tip x 25

9. Elution tube x 25

2. Reagent strip rack x 2

4. 3 mm/5 mm O -rings X 25

6. Sample tube x 25

8. Waste tank x 1

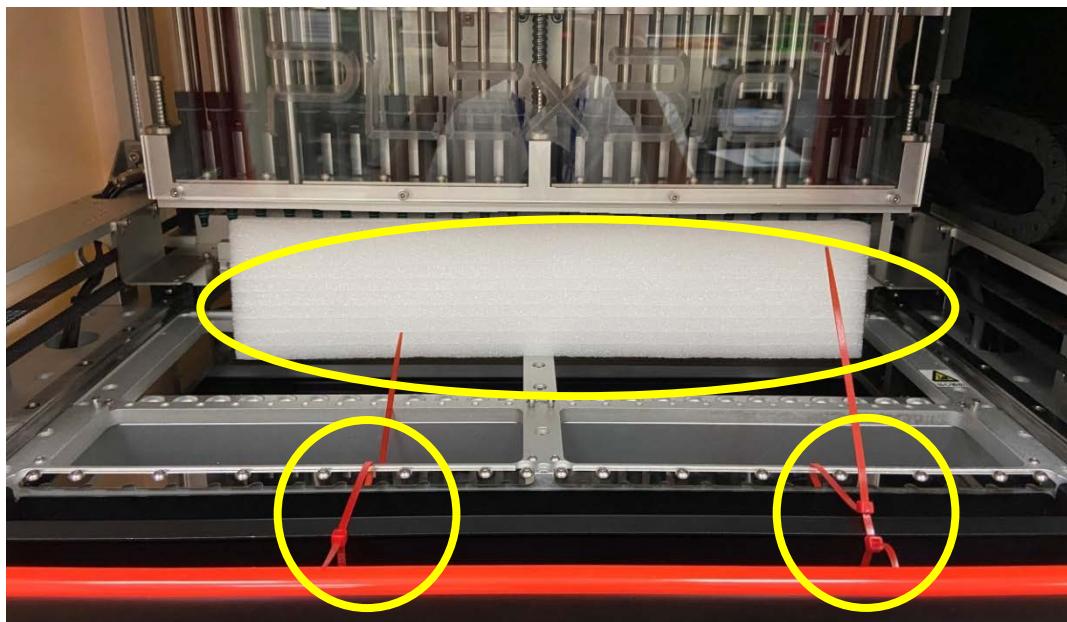
10. Grease x 1

3.2 System Installation

1 Before installation

Prepare an appropriate location and workbench that stable and strong enough to support the total weight of the equipment and keep adequate space for instrument ventilation.

2 Carefully cut and remove the two red cable ties from the waste tank

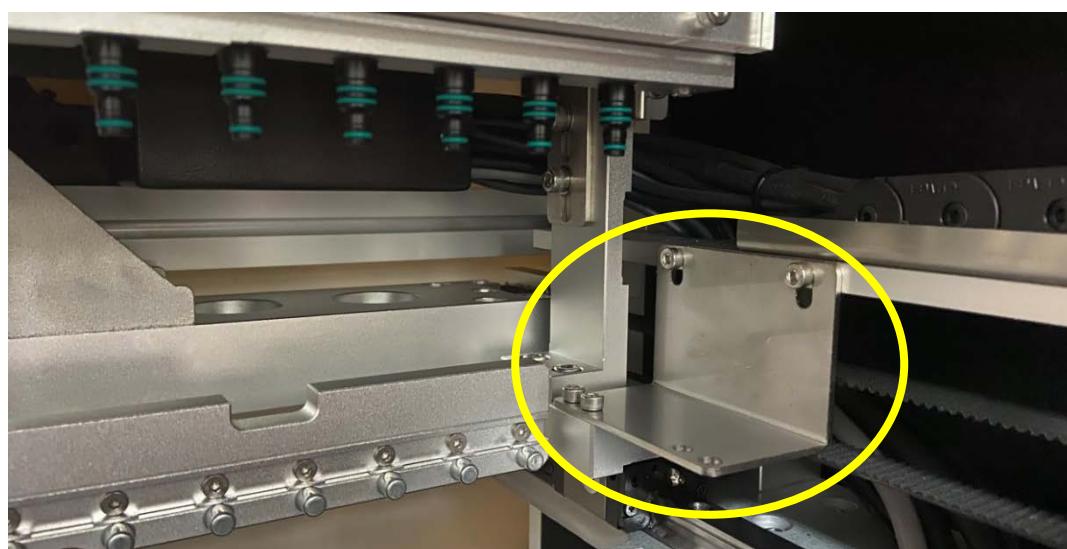


3 Remove the Styrofoam from the pipette module

Carefully remove the Styrofoam under the pipette module. Check the 3 O-rings to ensure they are installed on each pipette head.

4 Remove the right bracket with a 2.5mm hex key

Carefully remove 4 screws from the right bracket.



5 Remove the left bracket with a 2.5mm hex key

Carefully remove 4 screws from the left bracket



6 Remove the central bracket with a 2.5mm hex key

Carefully remove 2 screws from the central bracket



7 Plugin and turn on the machine.

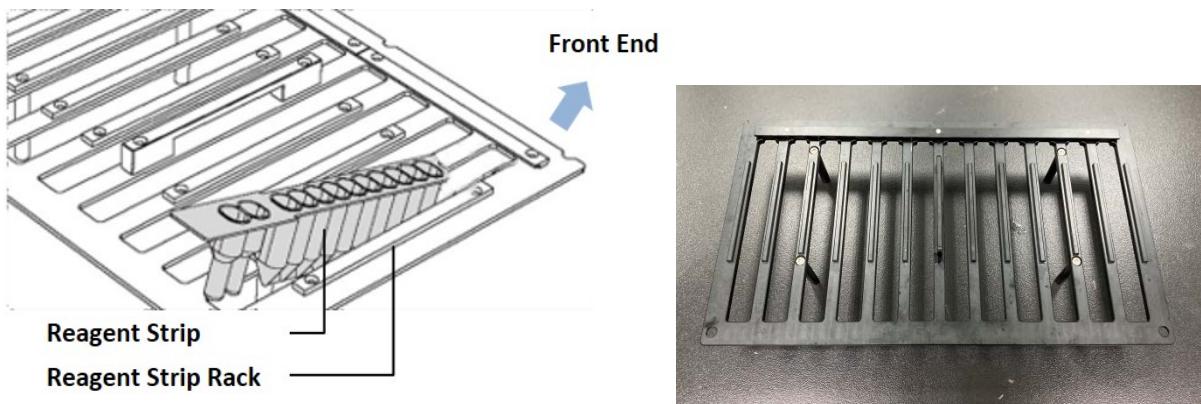
It will take a few minutes to finish the self-test. Do not open the door while the instrument is running self-test.

Chapter 4: Starting Extraction

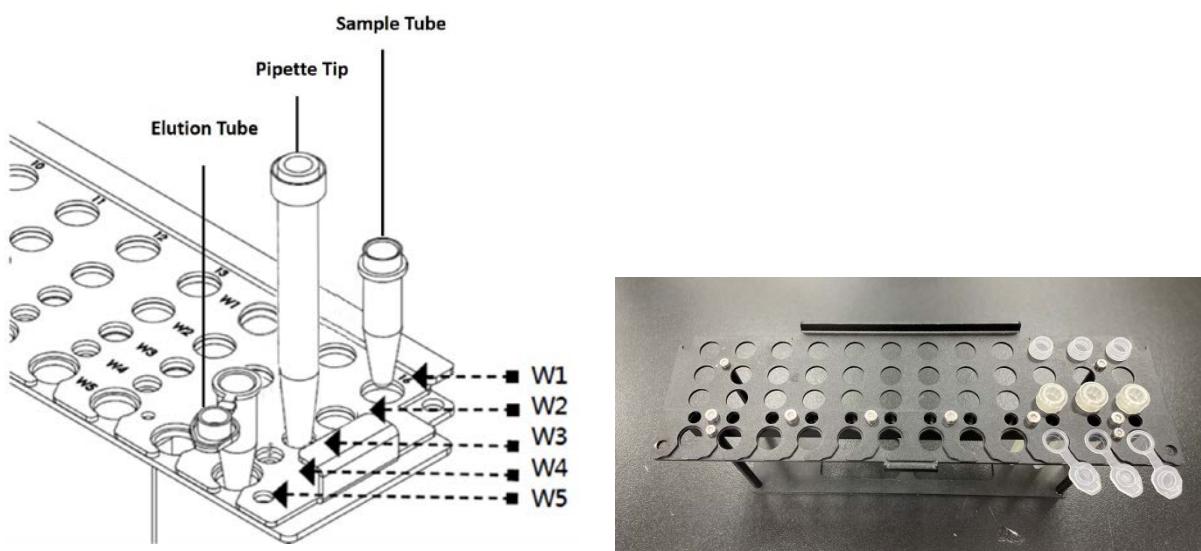
4.1 Run Extraction Program

1 Put the Reagent Strip into the Reagent Strip Rack

The front end of the Reagent Strip should be inserted into the slit of the Reagent Strip Rack.



2 Put the Extraction Kit Accessories into the Sample Rack



Follow the instruction of the extraction kit to place the prepared Sample Tube, Pipette Tip, and Elution Tube into the Sample Rack.

3 Put the loaded Reagent Strip Rack into the system

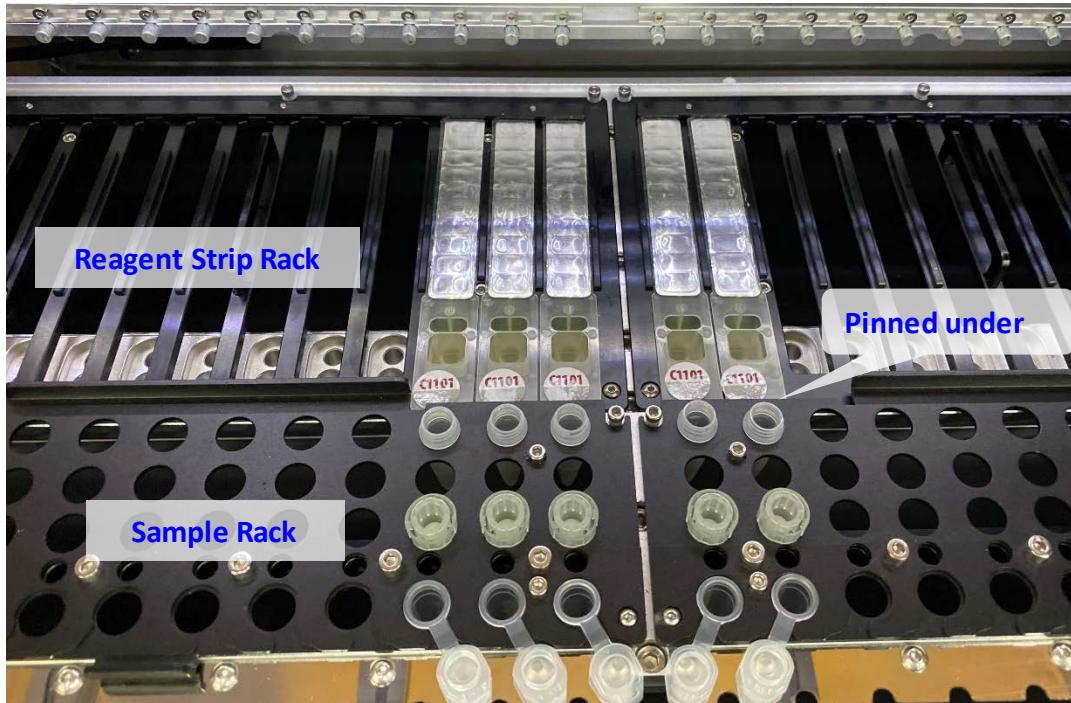
The Reagent Strip Rack should be placed as shown in the picture below. Note: the front end must be facing inward.



4 Put the loaded Sample Rack into the system

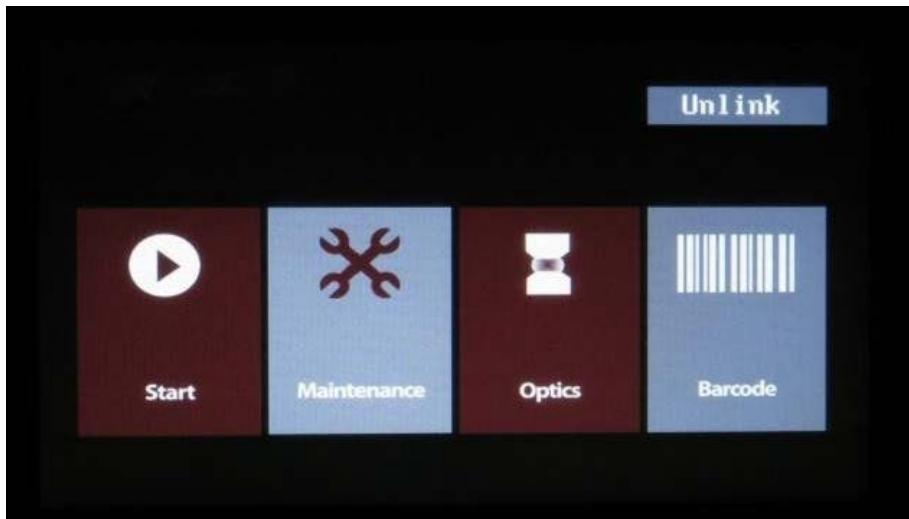
The Sample Rack should be placed as shown in the picture below. The edge of the Reagent Strip will be pinned under the Sample Rack.

IMPORTANT: The position of the loaded Reagent Strip in the Reagent Strip Rack and the loaded Sample Rack (loaded with Sample Tube, Pipette Tip, and Elution Tube in the depicted example) must align as shown in the picture.



5 Start the extraction program

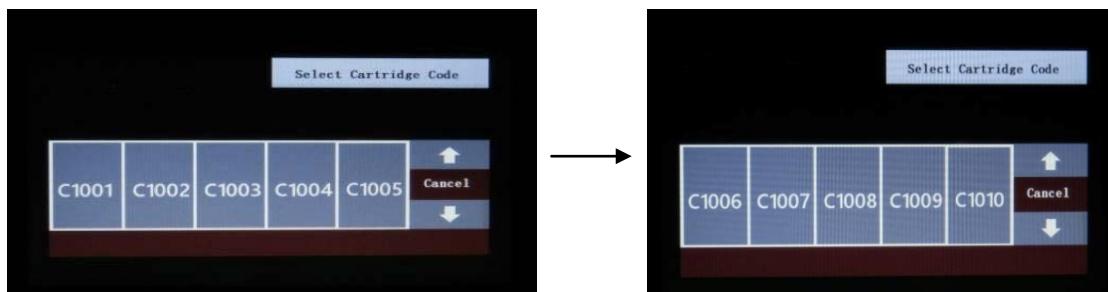
- (1) Follow the user manual of the extraction kit to set up the program. Click “Start” on the touchscreen.



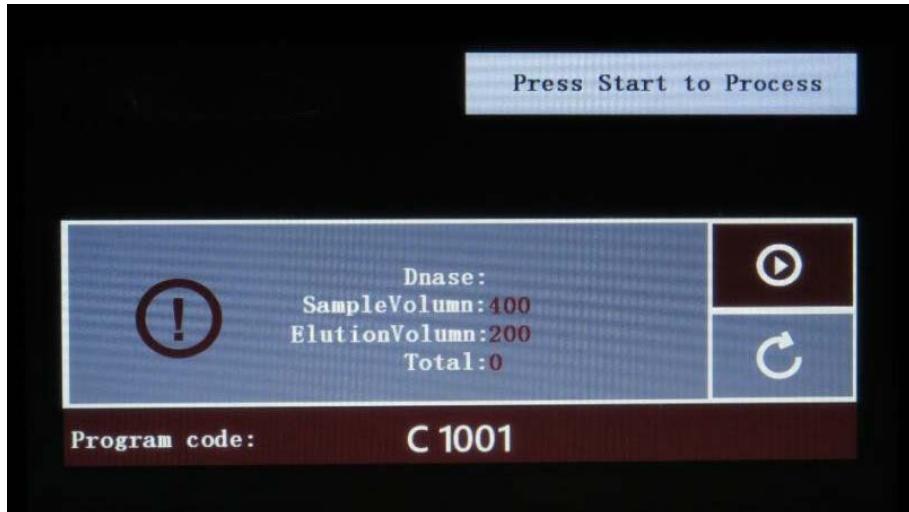
- (2) The program code is provided in the user manual of the extraction kit. Additionally, for some assays, the program code may also be found on the Reagent Strip (see picture below).



- (3) Select the required program code (to find the program, click the up or down arrow to view different pages).



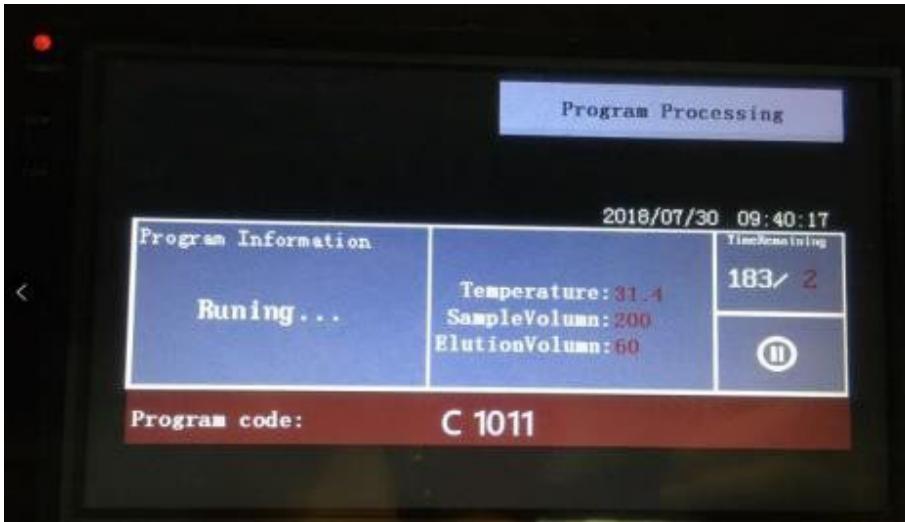
- (4) Set up the program conditions following the instruction of extraction kit.
- (5) Make sure all parameters selected are correct and press  to start the program.



- (6) The touchscreen will display “Finish” when the program completes. Collect the Elution Tube containing the extracted nucleic acids and store it properly.
- (7) Discard the used Reagent Strip, Tubes, and Pipette Tips.
- (8) Discard the waste from the waste tank and clean the tank after each run (only required for certain kits.)
- (9) Decontaminate the system by performing a UV sterilization program. See **Chapter 5: Maintenance** for details.

4.2 Emergency Stop

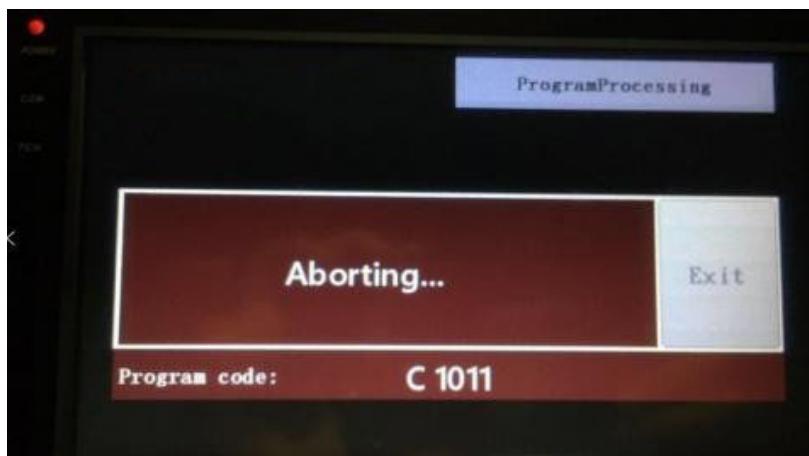
- 1 To pause the extraction process, press the  button.
- 2



- 2 To abort the process, press "Abort" after a pause. To continue the process, press "Continue" after a pause.

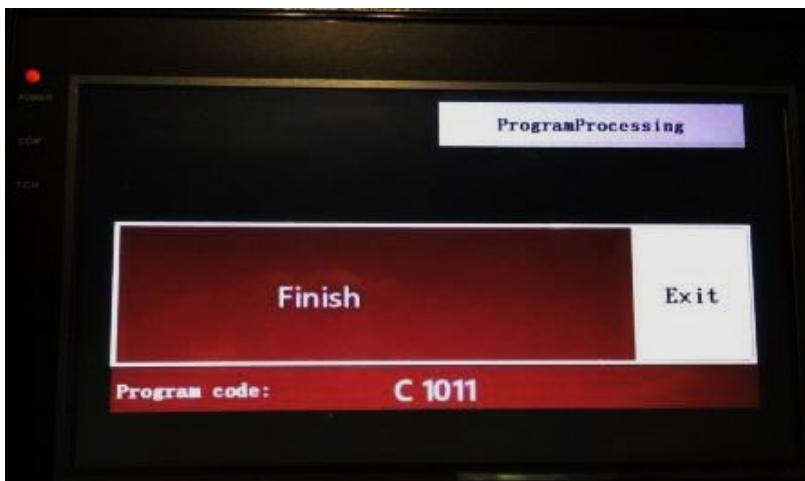


- 3 When the program is aborted, the instrument will move all modules back to the original



position. Please do not touch the instrument during the aborting process.

- 4 After the process is completed, the screen will show Finish. Click “Exit” to return to the main menu.

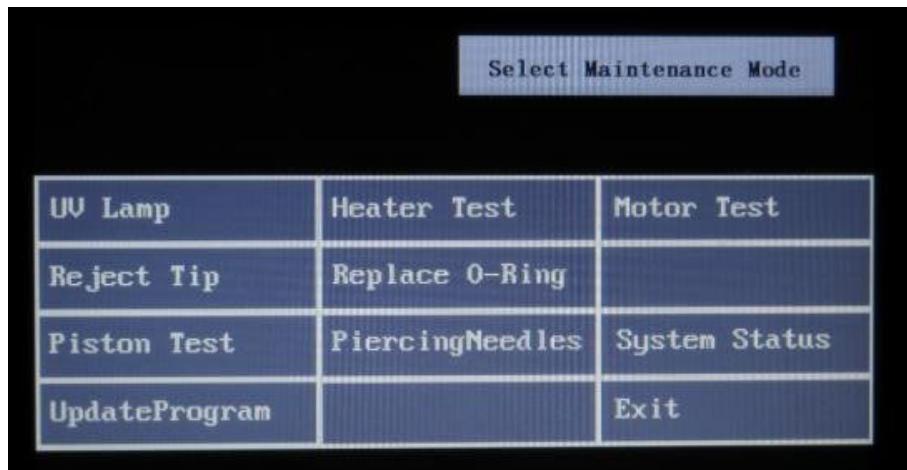


Chapter 5: Maintenance

User maintenance includes UV sterilization, cleaning, Heater and Motor testing, and other procedures. Please perform maintenance regularly to ensure optimal system performance.

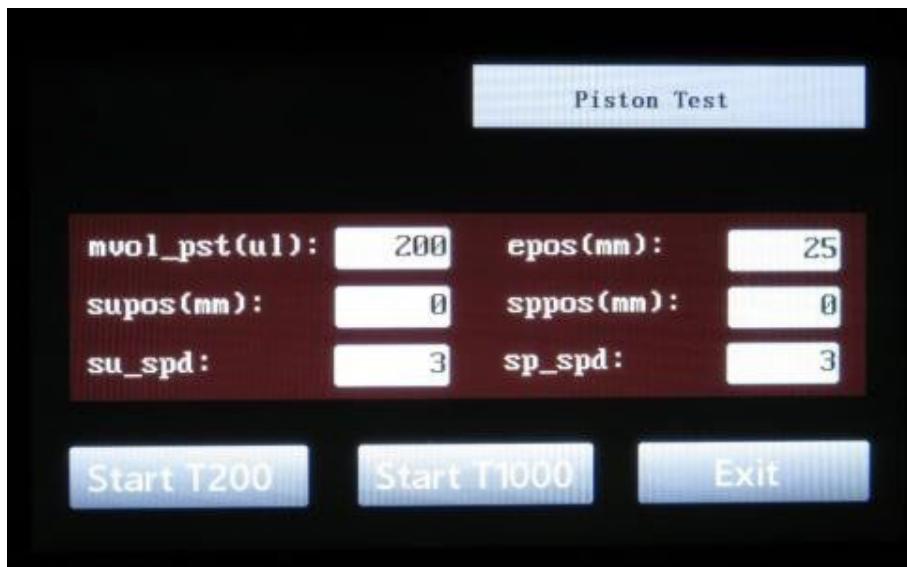
1 Replace O-ring

Select “Replace O-Ring” and use the tool to remove the old O-ring. Replace with a new O-ring. Lubricate it and run piston test



2 Piston test

Select “Piston Test”. Select “Start T200” or “Start T1000” to start the test.

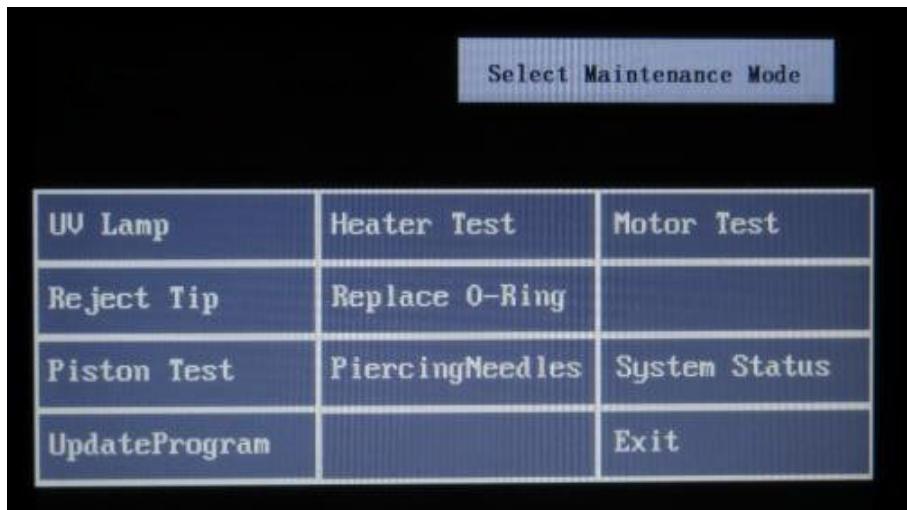


Note:

Based on the frequency of use, it is recommended the user conduct a piston test and lubricate O-ring once a month.

3 Cleaning the Piercing Needle

Select “Piercing Needles”. Wipe the dirt piercing needle with 70-75% alcohol.



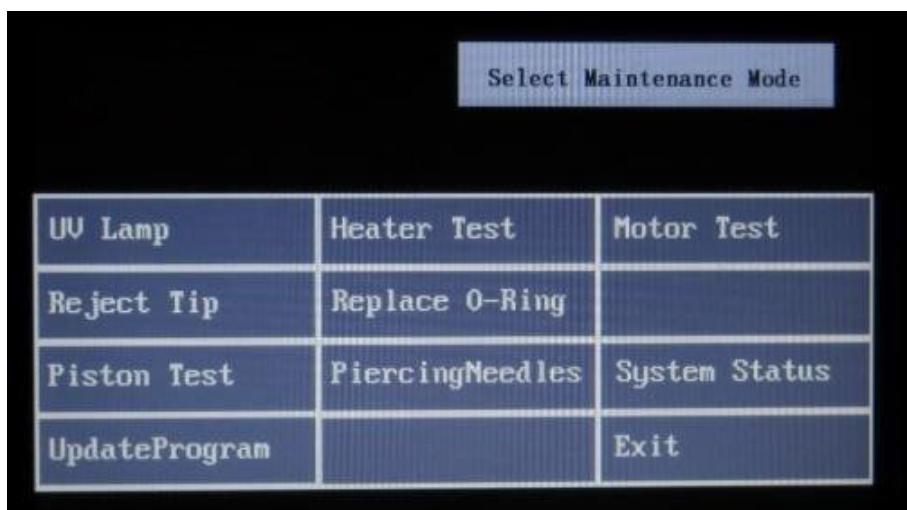
Be careful when cleaning the Piercing Needle. The needle tip is very sharp.

4 Remove the tips

When the system stops abnormally due to power failure or other reasons, the tip might be stuck on the piston. Select “Reject Tip” and remove the tips manually.

5 Heater test

Before running a heater test, add 840 μ l water into the large wells and small wells of Reagent Strips. Then, put the Reagent Strip into Reagent Strip Rack and place a calibrated thermometer in the water. Select “Heater Test”



Select “Start” to start the test

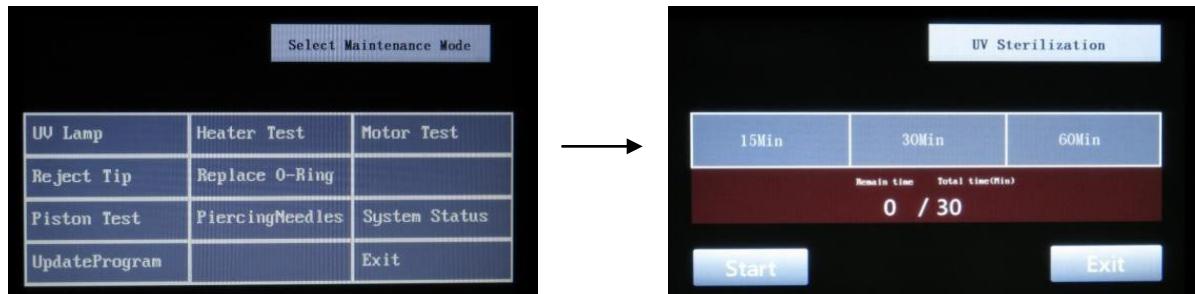


The heater will start to heat up to 65°C. Check whether the thermometer temperature matches with the temperature displayed on the screen.

The acceptable temperature range is $65 \pm 5^\circ\text{C}$

6 UV sterilization

Select “UV Lamp” then select the desired sterilization period.

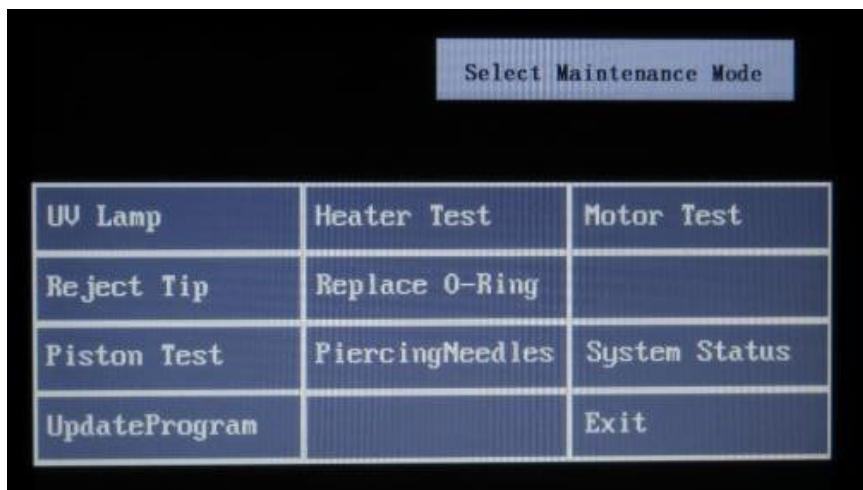


UV light is harmful to the human eye. Do not look into the instrument when performing UV sterilization.

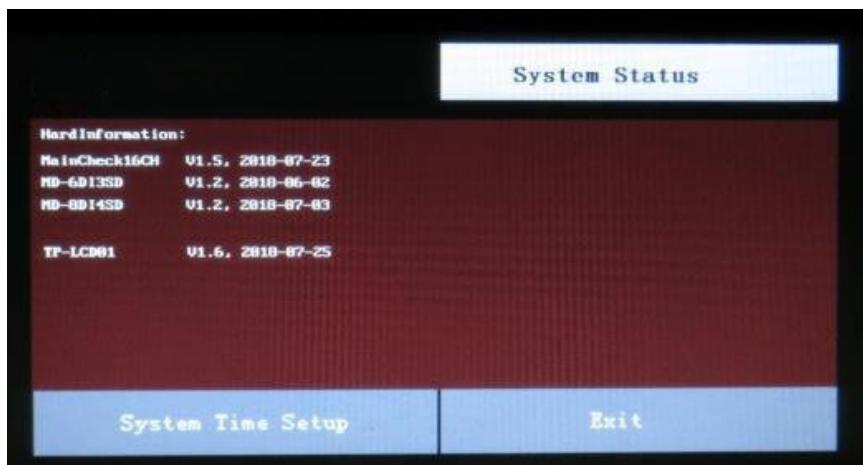
7 System status

You can check the system status and set the system time.

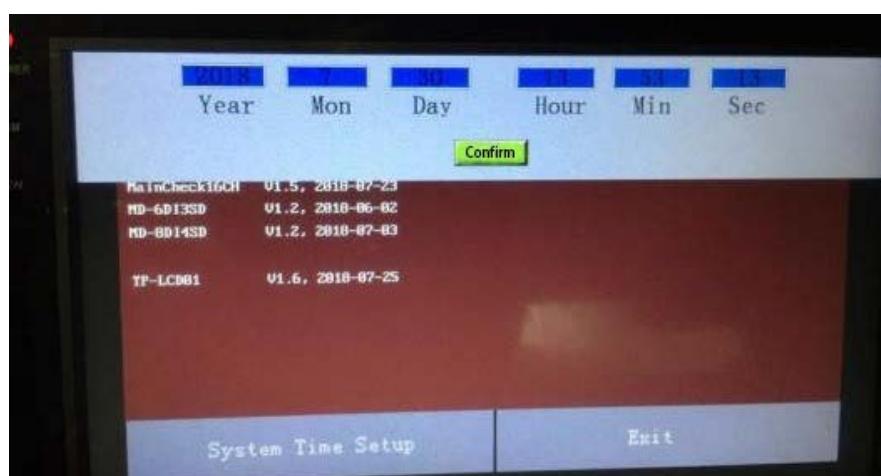
(1) Select “SystemStatus”



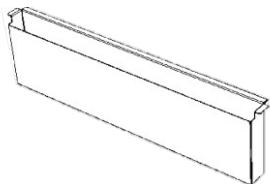
(2) Select “System Time Setup” and set the system time.



(3) Set system time (local date and time) then press “Confirm” (green) button to save the change.

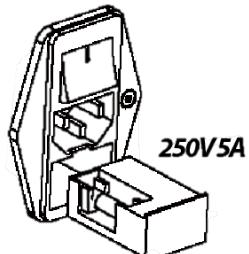


8 Cleaning the waste tank



When the extraction program is completed, please discard the waste, and clean the waste tank after each run.

9 Replace the fuse



Use the specified fuse as indicated to avoid the possible risk of fire or electric shock.

Chapter 6: Getting Help

PlexBio Co., Ltd.

6F-1, No. 351, Yangguang St., Neihu District
Taipei City 11491, Taiwan

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IntelliPrep Nucleic Acid Extraction Kit

REF

83003 96 Preparations



Manufactured For:

PlexBio Co., Ltd.
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Taipei City, 11491, Taiwan
TEL: + 886-2-2627-5878
<http://www.plexbio.com>



IMPORTANT:

Read the instructions carefully prior to use

1. INTENDED USE

The IntelliPrep Nucleic Acid Extraction Kit is a collection of reagents intended to be used with the IntelliPrep Automated Nucleic Acid Extraction System for isolation and purification of nucleic acid from nasopharyngeal (NP), oropharyngeal (OP), anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) specimens. The product is intended to be used by professional users who are trained in molecular biological techniques. The IntelliPrep Nucleic Acid Extraction Kit is only for use under the FDA's Emergency Use Authorization.

2. TECHNOLOGICAL PRINCIPLES

Important steps for the nucleic acid extraction process include:

- Lysis of sample material, releasing the nucleic acids.
- Binding between the nucleic acids and the beads under the conditions of the lysis buffer.
- Magnetic separation of the bead along with bonded nucleic acids from the lysed sample.
- Removal of unbound substances such as proteins, cell debris, and PCR inhibitors by washing.
- Elution of the purified nucleic acids from the beads.

3. WARNINGS AND PRECAUTIONS

- For Emergency Use Authorization Only.
- For *In Vitro* Diagnostic Use Only (IVD).
- For Prescription Use Only (Rx).
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- This product should be used by qualified laboratory personnel only.
- ~~Do not use a kit or reagent past its expiration date.~~

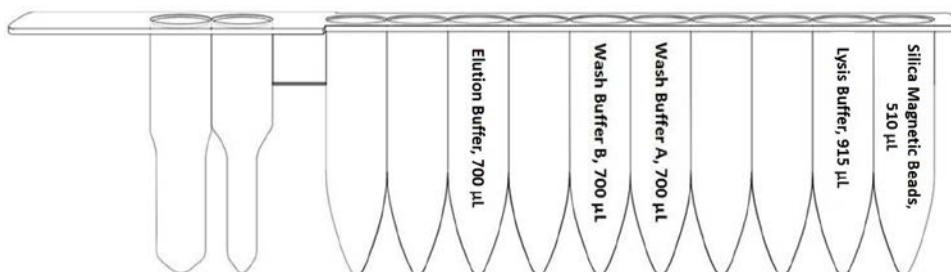
- Sample preparation should be performed according to approved guidelines such as those published by Clinical And Laboratory Standards Institute; clean all equipment and surface areas regularly (e.g. The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution).
- Do not dilute the component reagents. The reagents have been diluted optimally.
- All chemicals, biological materials and human origin samples should be considered as potentially hazardous and/or infectious and should be treated accordingly.
- Some reagent contains hazardous compounds. Follow Good Laboratory Practices and Universal Precautions guidelines to avoid any risk.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens, and waste according to applicable central/federal, state, and local regulations.
- General laboratory precautions should be taken:
 - Do not pipette by mouth.
 - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
 - Do not eat, drink or smoke in the laboratory.
 - Wash hands thoroughly after handling samples and reagents.
- **Avoid RNase contamination:**
 - Create an RNase-free working environment.
 - Wear gloves during all steps of the procedure.
 - Change gloves frequently.
 - Use only certified RNase-free sterile, disposable polypropylene tubes and filter strips.
 - Keep tubes closed whenever possible during the preparation.
 - Use RNase removing product to clean bench surfaces, pipettes and other components used in the experiment.
- Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

4. PRODUCT USE LIMITATIONS

- Reliable results depend on proper sample collection, transportation, storage, and processing.
- This product should be used by trained personnel following the protocol described in this manual. Any deviation or violation may compromise the performance, affecting the result of downstream applications.
- This product is validated using the specimen material specified in the user manual. Using other specimen materials may compromise the performance, affecting the result of downstream applications.
- This product is validated using sample medium specified in the user manual. Using other sample medium or insufficient number of cells may compromise the performance, affecting the result of downstream applications.

5. REAGENT STRIP CONTENTS

The IntelliPrep Nucleic Acid Extraction Kit contains 96 Reagent Strips. Each Reagent Strip is prefilled with multiple reagents as shown in the figure below:



6. APPLICABLE MODELS:

IntelliPrep Automated Nucleic Acid Extraction System (PlexBio; Cat. No. 80040)

7. STORAGE, STABILITY AND TRANSPORTATION

Storage

All kit components should be stored at 15-30°C.

Stability

Do not use any kit that has expired. All components are stable up to the expiration date on the label if handled and stored under the recommended conditions.

Transportation

The shipping temperature for the kit is 15-30°C. Visually inspect the Reagent Strips upon receiving the package. If the kit package or components are incomplete, or if any of the component(s) is damaged, do not use it and please contact PlexBio Customer Service (service@plexbio.com).

8. MATERIALS REQUIRED BUT NOT SUPPLIED

Required products for compatibility with IntelliPrep Nucleic Acid Extraction Kit:

- IntelliPrep Automated Nucleic Acid Extraction System (PlexBio; Cat. No. 80040)
- Extraction Kit Accessories (PlexBio; Cat. No. 80027), which includes
 - Pipette tips and holder set
 - Sample tubes
 - Elution tubes
- Disposable gloves, powder-less

9. SPECIMENS

Users must verify the storage conditions (such as temperature and time) of the specimens. Make sure the starting materials meet specified requirements.

Nasopharyngeal swab

Follow manufacturer's instructions of specimen collection device for proper collection and storage methods. Only synthetic fiber swabs with plastic shafts should be used. Do not use calcium alginate swabs or swabs with wooden shafts (which may inactivate some viruses and inhibit PCR testing). Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media. Recommend extracting nucleic acids freshly.

10. ASSAY PROCEDURE

Read the instructions thoroughly and strictly follow every step of the protocol.

Please also refer to the User Manual of the IntelliPrep Automated Nucleic Acid Extraction System

1. Before start, turn on the power of IntelliPrep Automated Nucleic Acid Extraction System and warm up for 20 minutes.
 2. Take out the **Reagent Strip Rack** and the **Sample Rack** from IntelliPrep Automated Nucleic Acid System.
 3. Put the **Reagent Strip** into **Reagent Strip Rack**. The front end of the **Reagent Strip** (Fig. 1) should be inserted into the slit of the **Reagent Strip Rack** (Fig. 2). Each specimen requires one **Reagent Strip** for extraction.
 4. Transfer 400 μ L specimen into the **Sample Tube**.
- Note:** Use the **Sample Tube** provided in the Extraction Kit Accessories (PlexBio; Cat. No. 80027).
5. Place the prepared **Sample Tube**, **Pipette Tip**, and **Elution Tube** in **W1**, **W3**, and **W5** of **Sample Rack** respectively (Fig. 3).
 6. The position of the loaded **Reagent Strip** in the **Reagent Strip Rack** and the **Sample Tube**, **Pipette Tip**, and **Elution Tube** in the **Sample Rack** needs to be aligned. Each specimen requires one set of **Sample Tube**, **Pipette Tip**, and **Elution Tube**.
- Note:** Use the **Pipette Tip** and **Elution Tube** provided in the Extraction Kit Accessories (PlexBio; Cat. No. 80027). The **Elution Tube** should remain uncapped.

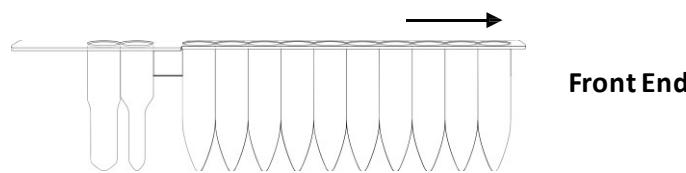
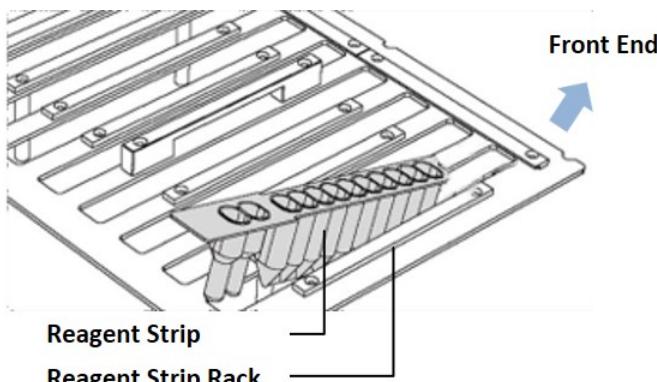


Fig. 1. Reagent Strip



Reagent Strip
Reagent Strip Rack

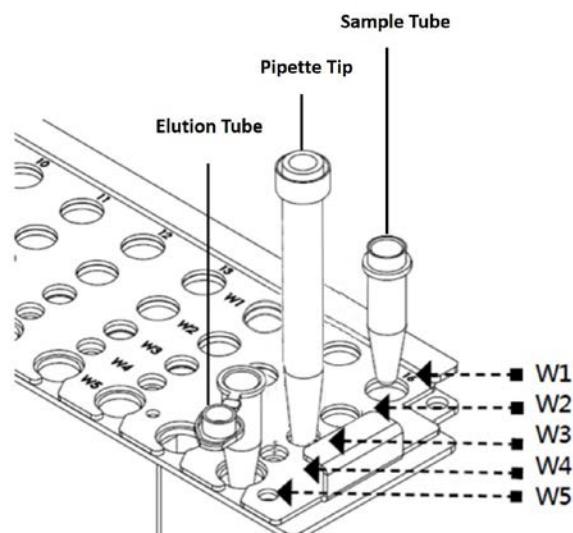


Fig. 2. Reagent Strip and Reagent Strip Rack

Fig. 3. Well Position of Sample Rack

7. Put the loaded **Reagent Strip Rack** into IntelliPrep Automated Nucleic Acid System.
8. Put the loaded **Sample Rack** into IntelliPrep Automated Nucleic Acid System. The edge of the **Reagent Strip** shall be pinned under the **Sample Rack**.
9. Close the front door of IntelliPrep Automated Nucleic Acid System.
10. Start the extraction program by selecting on the touchscreen: Click “Start” → Select “C1105” for program code → Select “400” for sample volume → Select “50” for elution volume.
11. Confirm the program selected and click “START”. The program will complete in about 1 hour.
12. After the program completes, collect the Elution Tube containing the extracted nucleic acids and store properly.
13. Discard the used **Reagent Strip**, **Sample Tube**, and **Pipette Tip**.

14. Decontaminate the instrument by selecting on the touchscreen: Click “Maintenance” → Select “UV” → Select “30 min” → Click “START”. Turn off the power after the program completes.

11. TROUBLESHOOTING

The troubleshooting listed below addresses possible problem causes and solutions provided during assay procedures.

Problem	Possible Cause	Recommendation
The extracted nucleic acids contain residual magnetic beads.	<ol style="list-style-type: none"> 1. The magnetic stand does not function properly. 2. Magnetic beads aggregation due to viscosity of the sample. 	<ol style="list-style-type: none"> 1. Contact PlexBio for instrument maintenance. 2. Use Normal Saline or PBS to dilute the specimen and extract the sample again.
The quality/ quantity of the extracted nucleic acids is low.	<ol style="list-style-type: none"> 1. Sample degraded or impure. 2. The reagents have lost reactivities. 	<ol style="list-style-type: none"> 1. Resample and make sure the starting materials meet specified requirements. 2. Make sure the kit is stored following the recommended conditions. Do not use expired kits.
The volume of the extracted nucleic acids is not enough.	<ol style="list-style-type: none"> 1. Pipette Tip clogged due to viscosity of the sample. 2. The instrument does not function properly. 	<ol style="list-style-type: none"> 1. Use Normal Saline or PBS to dilute the specimen and extract the sample again. 2. Contact PlexBio for instrument maintenance.
Cross-contamination	<ol style="list-style-type: none"> 1. Sample contaminated when being transferred manually. 2. Environment or equipment contaminated. 	<ol style="list-style-type: none"> 1. Pipet carefully and avoid passing other tubes from above. If the gloves contact the specimen or any reagents accidentally, discard the gloves immediately. 2. Before start, use bleach to wipe clean and decontaminate the work area and the surface of equipment. Wait 15 minutes and wipe clean everything with 70% alcohol twice. Decontaminate the instrument by running the UV program.

12. Symbols

Symbol	Explanation	Symbol	Explanation
	Batch number		Catalog number
	Manufacturer		Consult instructions for use
	Temperature limitation		Use by Date
	Contains sufficient for <n> tests		Caution
			Date of Manufacture

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PlexBio™ 100 Fluorescent Analyzer

User Manual

PlexBio Co., Ltd.



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- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
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Chapter 1. Introduction

1.1 Overview

Before using PlexBio™ 100 Fluorescent Analyzer, read this manual carefully. It provides essential hardware and software information regarding of PlexBio 100 Fluorescent Analyzer. Information in this manual includes:

- Safety information
- Symbols and Regulatory Labels
- System overview
- Hardware installation
- DeXipher™ software installation
- Starting DeXipher™ software and User Interface
- Reading IntelliPlex™ Assay Kit
- Reading Custom Assay (Quick Read, Quantitative and Qualitative assays)
- Manage saved files, results and reports
- Creating and applying protocols
- Maintenance and Calibration
- Settings
- Troubleshooting

1.2 Symbols and Regulatory Labels

There are important symbols used on the PlexBio 100 Fluorescent Analyzer and throughout this manual with different meanings, including identification, conditions, and safety warnings.

		
General cautions and warnings	Caution: pinch point	Warning: Biohazard
Manufacturer	 UL Mark	Consult instructions for use
		 Waste Electrical and Electronic Equipment
Federal Communications Commission (FCC) Mark	For Research Use Only	

Table 1. List of labels



General cautions and warnings

This symbol indicates a need for caution. Failure to follow instructions associated with this sign may result in possible damage to property or equipment, personal injury or death. Read and understand all warnings and cautionary safety instructions below before proceeding with the operation of this system.

- Inspect all components prior to use and immediately report any damage or defects observed to PlexBio Co., Ltd. or your local distributor.
- Contact PlexBio Co., Ltd. or your local distributor to schedule installation, maintenance or repairs of the system.
- PlexBio 100 Fluorescent Analyzer contains optical and electronics modules. Handle with extreme care when moving or relocating the system as mishandling can damage it.
- Lift the unit from the base only. DO NOT lift by or put excessive force on the plastic case.
- DO NOT put excessive force on the stage (plate holder) or it may become tilted or bent. This will severely interfere with the performance of the system.

- To avoid electric shock or possible damage to the system:
 - DO NOT open or remove the covers on the analyzer or system peripherals.
 - Use only power cords supplied with the system or cords with grounded outlets properly rated for the system.
 - Ensure the power cord is connected to properly grounded AC outlets only. Use of surge protector is recommended.
 - Users should not perform any maintenance or cleaning of the electrical components in the system.
- If any malfunction happens, turn off the power button and then disconnect the power cable.
- DO NOT look directly at the green LED light. When users open the lid, the shutter should turn the light off. If a malfunction occurs, contact PlexBio Co., Ltd. or your local distributors for servicing.



※ **Caution: Pinch point**

Do not put your hands under the sides of the lid when closing it.



※ **Warning: Biohazard**

Human and animal samples in the 96-well plate may contain biohazardous, infectious agents. To prevent spilling, wear gloves and handle with extreme care when loading the plate onto the stage (plate holder).



Fig. 1. The position of warning labels



Waste Electrical and Electronic Equipment

Within the European Union, the Waste Electrical and Electronic Equipment Directive 2002/96/EC requires that you properly dispose of electrical and electronic equipment when it reaches the end of its lifecycle.

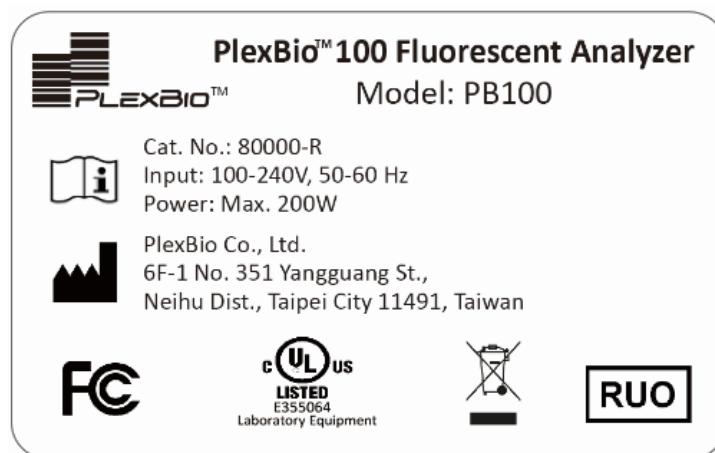


Fig. 2. PlexBio™ 100 Fluorescent Analyzer label on the backside of the instrument

Chapter 2. PlexBio™ 100 Fluorescent Analyzer System Overview

2.1 Product Description

PlexBio 100 Fluorescent Analyzer is a digital microscope system intended for fluorescence detection applications. The system includes a charge-coupled device (CCD) camera, a light-emitting diode (LED), a microscope, and a fluorescence detection unit and is operated by the software DeXipher™ (see chapter 4 for details). Image patterns and fluorescence signals of πCode™ MicroDisc can be obtained by analyzing the images of πCode™ MicroDisc captured by PlexBio™ 100 Fluorescent Analyzer.

2.2 πCode™ MicroDisc Technology

PlexBio's Precision Image Code (π Code™ = π Code™) MicroDiscs are manufactured using photolithography fabrication process which can generate over 16,000 distinct circular image codes for multiplexing applications. To be used in biological applications, each circular disc is encapsulated in highly stable biocompatible polymers with an added paramagnetic property which allow bio-conjugation, washing, and automation applications.

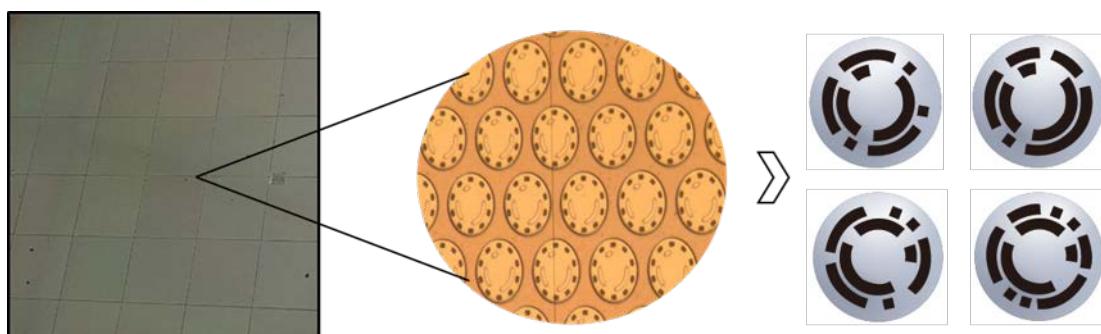


Fig. 3. Photolithography fabrication process of πCode™ MicroDisc

Each πCode MicroDisc has a distinct circular image pattern, which corresponds to the specific capture agents conjugated to the disc surface, thus allowing the capture and detection of specific analytes from samples. Virtually any probe can be conjugated to πCode MicroDiscs, including DNA, RNA, antigens, antibodies, proteins, or chemical compounds.

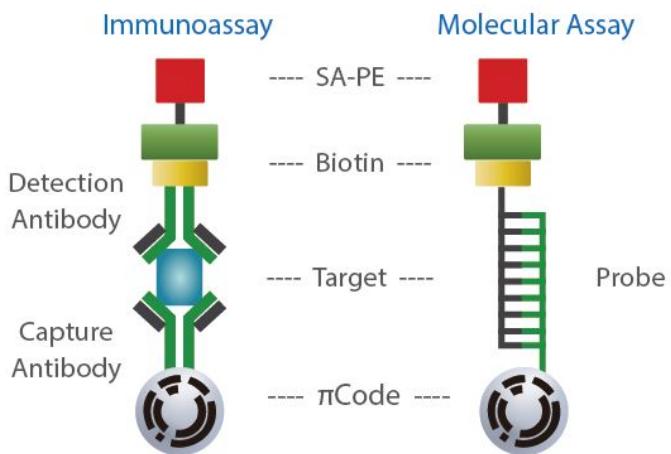


Fig. 4. Application of πCode™ MicroDisc for immunoassay and molecular assay

To perform an assay, πCode MicroDiscs with the desired detection probes are pooled together and added to a single well with corresponding sample, followed by hybridization and fluorescent labeling. Assay detection is accomplished by optical imaging through the PlexBio 100 Fluorescent Analyzer, which uses the CCD camera to read the distinct image patterns under bright field and qualitate/quantify the analyte by reading the fluorescence signal intensity under dark field, reporting the reactions occurring at each πCode MicroDisc in a user-friendly interface.

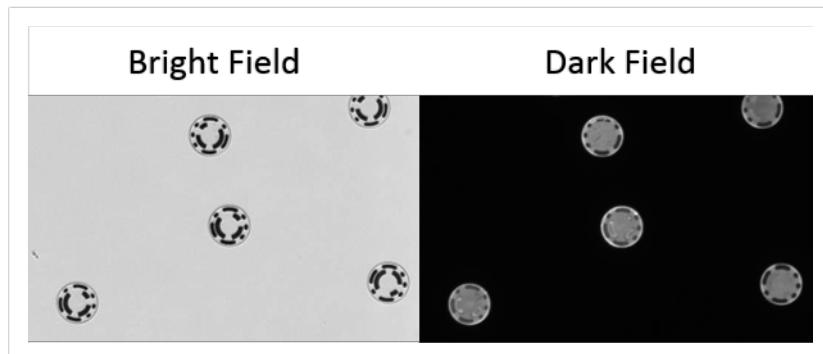


Fig. 5. Bright and dark field images after reporting

2.3 Features of PlexBio™ 100 Fluorescent Analyzer

The PlexBio 100 Fluorescent Analyzer is a robust optical imaging system that is highly effective and easy to use. The compact machine is easy to maintain (no fluid waste) and accurately decodes πCode MicroDiscs through high-contrast imaging. The system is compatible with any assay using PlexBio's multiplex-compatible πCode MicroDiscs.

Features:

- Easy to use: intuitive, user-friendly interface.
- Quick: within 15-minute system warm up; less than 50 seconds reading time per well.
- Robust: no-fuss maintenance due to simplicity of the instrument; no probes or fluids necessary.
- Accurate: πCode image pattern classification of only non-overlapping πCode MicroDiscs means virtually no misread πCode MicroDiscs. πCode MicroDiscs are read at the bottom of wells, eliminating cross-over risk.
- Efficient: run up to 96 tests in parallel; detect multiple targets per well and reduce sample volume by multiplexing assays.
- Flexible: use PlexBio's commercial assays or develop your own, with up to 16,000 unique πCode MicroDisc to mix and match.
- Traceable: original data can be traced back to individual πCode MicroDisc post-assay.

2.4 Instrument Specifications

Model	PB100
OPTICS	
Coding	Image Pattern
Optics (Excitation)	LED
Optics (Detection)	CCD imager
A/D resolution	14 bits
Focus Lens	10x magnification
OPERATION ENVIRONMENT	
Temperature	15 ~ 35 °C (59 ~ 95 °F)
Humidity	20 ~ 80% RH, non-condensing
Altitude	Up to 3,000 meters above mean sea level
STORAGE CONDITIONS	
Temperature	-10 ~ 70 °C (14 ~ 158 °F)
Humidity	10 ~ 80% RH non-condensing
PERFORMANCE	
Supported plates	96-well plate (Plexbio; Cat. No. 80025 or Greiner Bio-one; Cat. No. 655101)
πCode Classification Accuracy	≥ 98%
πCode Recognition Precision (CV)	≤ 2.5%
MFI Precision (CV)	≤ 1.5%
Daily Start-Up	≤ 15 min
Reading Time	1 well ≤ 50 sec
PHYSICAL CHARACTERISTICS	
Power	100-240V, 50-60Hz
Dimensions	27.3 cm W x 54 cm D x 44.1 cm H
Weight	25 kg (55.0 lbs)
Connection	USB and Ethernet

Table 2. PlexBio 100 Fluorescent Analyzer specifications

Chapter 3. Hardware Overview

3.1 Components

Note that the PlexBio 100 Fluorescent Analyzer is to be installed and serviced by PlexBio's qualified service engineers only. Contact PlexBio Co., Ltd. or your local distributor to schedule an installation, maintenance or any repair of the system.

The PlexBio 100 Fluorescent Analyzer includes:

- 1 PlexBio 100 system
- 1 USB 2.0 cable (A, B head)
- 1 Ethernet cable
- 2 AC power cords (110V, 220V)
- 1 Installation USB: includes DeXipher™ Basic software.
- 1 User Manual
- Optional items: 1 computer

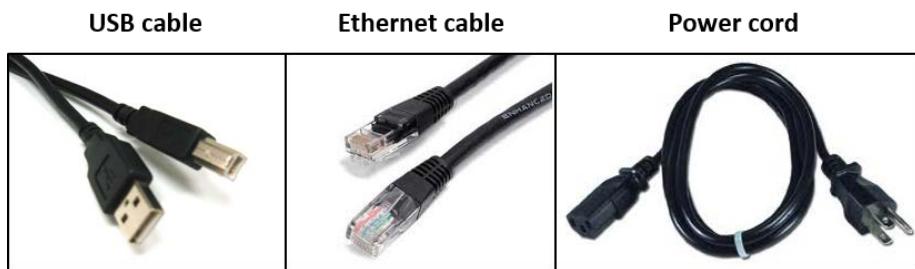


Fig. 6. Connecting accessories

Lift PlexBio 100 Fluorescent Analyzer from the bottom if you want to move the system. Do not pull, push, or bend the case from the side. The PlexBio 100 Fluorescent Analyzer needs an operation area of 0.20 square meters on a flat, stable and supportive bench. The system also needs to be placed at least 10 centimeters away from the wall for cable connection with PC and for efficient cooling.



※ Caution

- Under the following situations, contact PlexBio Co., Ltd. or local distributors for installation verification: Instrument installation; Computer replacement; Instrument relocation, or replacement of any components.
- After turning off the PlexBio 100 Fluorescent Analyzer, we recommend unplugging the power cable to fully disconnect the system from the main circuit. Do not position the equipment so that it is difficult to disconnect the plugs.

3.2 Minimum Computer Requirements

Component	Minimum	Recommended
Operation System	Microsoft Windows 7 Professional SP1 64bit	Microsoft Windows 10
Minimum screen resolution	1024 x 768 pixels	1024 x 768 pixels or higher
CPU	Intel core i7	Intel core i7 or above
Main Memory	4 GB DDR2 SDRAM	8 GB DDR2 SDRAM
Hard Drive	500GB 7500RPM SATA HDD	1TB 7500RPM SATA HDD
Port for connection	1 USB and 1 Ethernet port	1 USB and 1 Ethernet port or more
Network:	100MB Ethernet	100MB Ethernet or above
.NET Framework	4.0	4.0 or above
PDF Viewer	Adobe Reader 10.0	Adobe Reader 10.0 or above
Office Tools	-	Microsoft Office Compatible

Table 3. Computer requirements

3.3 Hardware Setup

Note that installation of the PlexBio 100 Fluorescent Analyzer is to be performed by PlexBio's qualified service engineers only.

1. Ensure the power button is off before connecting the power cable.
2. Connect USB cable and Ethernet cable between PlexBio 100 Fluorescent Analyzer and computer.
3. Connect PlexBio 100 Fluorescent Analyzer power cable.
4. Turn on PlexBio 100 Fluorescent Analyzer. The indicator light on the front will be steady pink during warm-up.
5. Warm-up takes about 15 minutes. The indicator light turns white, once the PlexBio 100 Fluorescent Analyzer is ready to use.

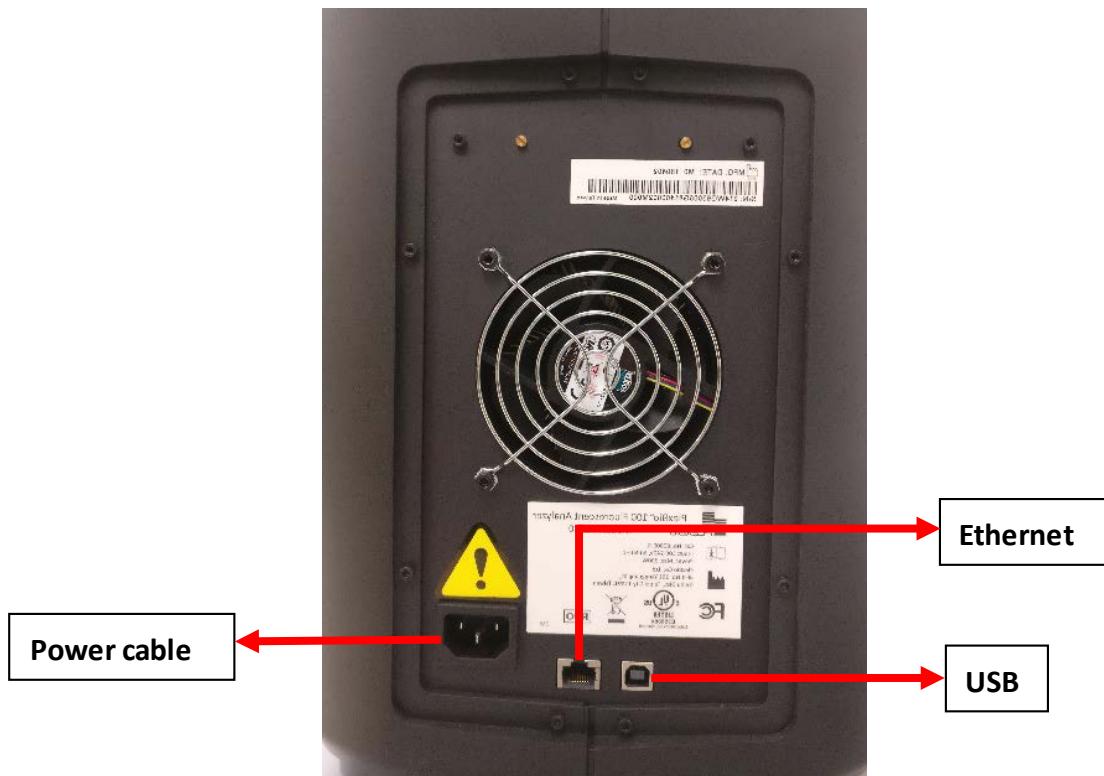
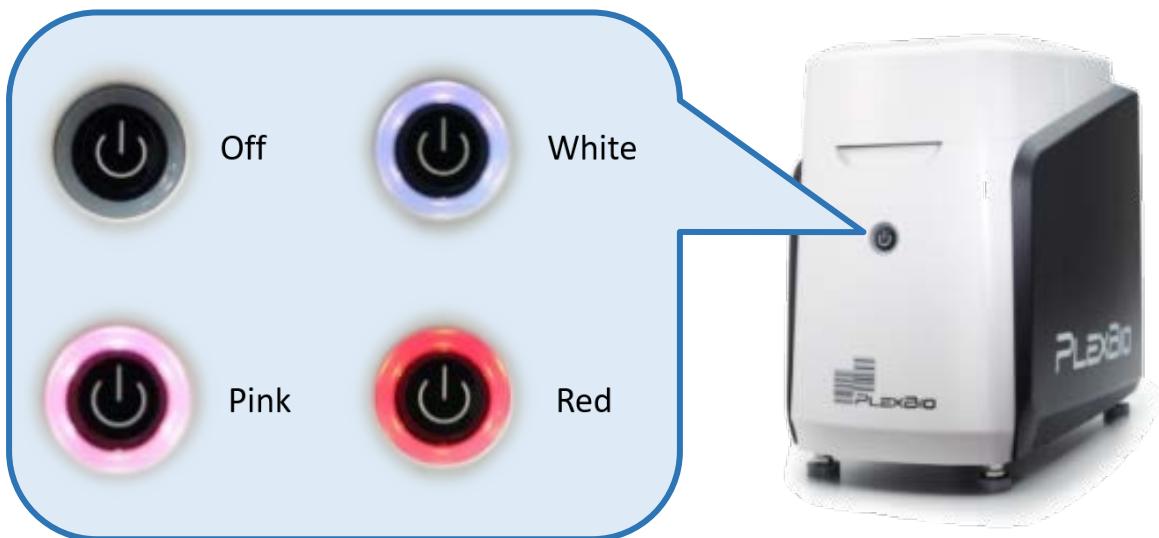


Fig. 7. PlexBio 100 Fluorescent Analyzer ports

3.4 Indicator Light Signals



Mode	Signals
Warm up	Steady Pink
Standby/Finished reading	Steady White
Reading	Alternating Flashing Pink and White
Lid open before reading	Flashing Pink
Lid open during reading	Alternating Flashing Pink and White with warning message in DeXipher software
Error	Flashing Red

Table 4. Indicator light signals

Chapter 4. DeXipher™ Software Overview

4.1 Software License Types

DeXipher™ Basic is included in every PlexBio 100 Fluorescent Analyzer purchase. DeXipher Basic has all the required functions for users to decode and analyze πCode MicroDiscs in a well of a 96-well plate. All results can be exported as raw data for further analysis.

For advanced functions such as data analysis, kit applications, and account management, contact PlexBio Co., Ltd. or local distributor to upgrade to DeXipher RUO. Feature comparison between DeXipher Basic and RUO is shown below.

Key Features	Basic	RUO
Number of Accounts #	1	>1
Account Management		✓
Quick Read	✓	✓
Export Raw Data	✓	✓
Identify Kit ID and Lot ID		✓
Calibration (Stage and Fluorescence)	✓	✓
Data and Results (Check Previous Data/ Summary Data)	✓	✓
Define Standard, Control, and Sample Well		✓
Create Your Own Protocol/ Assay		✓
Data Analysis and Reports (Standard Curve Calculation and Charts)		✓
Kit Reports (e.g. IntelliPlex-branded Assays)		✓

Table 5. Software license type comparison

4.2 Installation

Note that installation of the PlexBio 100 Fluorescent Analyzer is to be performed by PlexBio's qualified service engineers only.

DeXipher Basic will be pre-installed for all users purchasing the PlexBio 100 Fluorescent Analyzer and a computer as package. DeXipher can also be installed or upgraded during installation of the PlexBio 100 Fluorescent Analyzer by trained service personnel. A copy of DeXipher Basic is provided on the Installation USB included in the PlexBio 100 Fluorescent Analyzer package. Contact PlexBio Co., Ltd. or local distributor for more information about DeXipher RUO license.

Before Installation

Make sure that the connected computer meets the minimum specification and that the screen supports a resolution of at least **1024 x 768** pixels (refer to section 3.2). DeXipher User interface (UI) may not be displayed properly at a lower resolution.

Installation

To operate DeXipher successfully, DeXipher client and DeXipher Server must both be installed. DeXipher client is the software known as "DeXipher", which is used to set up assays, control PlexBio 100 Analyzer, and obtain analysis results, whereas DeXipher Server manages the database of saved files and results. DeXipher supports installation of standalone or network configuration.

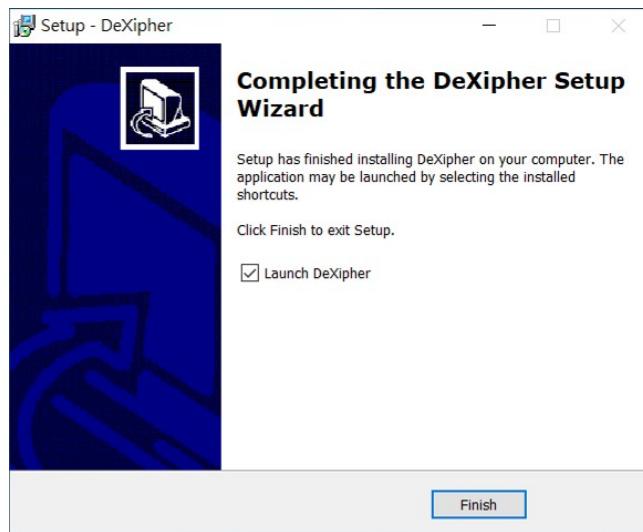
The standalone configuration runs independently from any network and requires installation of DeXipher client and DeXipher Server on the same computer, refer to section 4.2.1 for more information.

In the network configuration, several DeXipher clients can be connected to one central DeXipher Server. DeXipher client must be connected to DeXipher Server to be operational. All DeXipher client copies have the same license type as DeXipher Server. Refer to section 4.2.2 for more information on how to setup and use a network.

4.2.1 Installation of Standalone DeXipher Configuration

The standalone configuration requires installation of DeXipher client and DeXipher Server on the same computer.

- I. Install DeXipher Server by running “DeXipher_Server” in the DeXipher folder and follow the on-screen instructions. Install all components of the DeXipher Server.
- II. Install DeXipher client by running “DeXipher” in the DeXipher folder and follow the on-screen instructions. Once installation is completed, click “Finish” to close the application.



If asked, allow App/software to make changes to Windows.

If a firewall is active, grant all components during the installation process access.

Ensure all components are installed before using DeXipher with the PlexBio 100 Fluorescent Analyzer.

4.2.2 Installation of DeXipher Network Configuration

The network configuration has DeXipher client(s) and DeXipher Server installed on separate computers. After installation, **DeXipher client will only be operational if connected to DeXipher Server.**

The network installation offers the ability to share the same database between all DeXipher clients connected to the DeXipher Server. This allows setting up and creating new assays or protocols on any computer with DeXipher client installed and share with all DeXipher clients connected in the same network. The created assays can only be run on a DeXipher client that is connected to a PlexBio 100 Fluorescent Analyzer while results are shared with all DeXipher clients in the network.

Note that **IntelliPlex Assay** (DeXipher RUO only) files require the assay-specific APP to be installed on the same computer as the DeXipher client software (refer to section 6.1) to access reports (see section 9.4). DeXipher clients that do not have the APP installed locally will not be able to open reports. The APP does not need to be installed with the DeXipher Server.

The assay-lot-specific ENC file (see section 12.4) only needs to be imported once and will be shared with all DeXipher clients in the network.

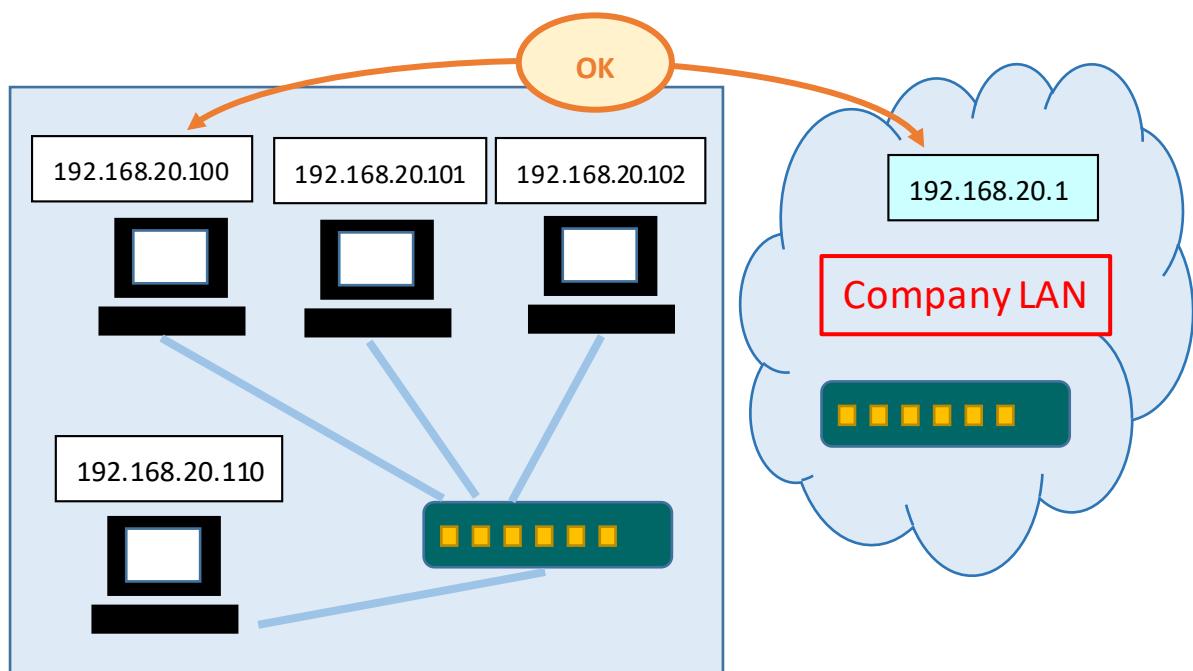
Note: Created assays, protocols and results are shared with all DeXipher clients in the same network. Access to information is subjected to the permission settings (refer to section 9.6 for permission related to files and results and chapter 10 for permission settings related to protocols).

DeXipher Network Server Configuration

The DeXipher Network can be setup as a separate LAN (Local Area Network), keeping the DeXipher Network separate from other networks. The DeXipher Network can also be integrated into the larger network ensuring connectivity between all computers and devices. For network setup we recommend using router, hub or switch of at least 1 Gbps (1,000 Mbps).

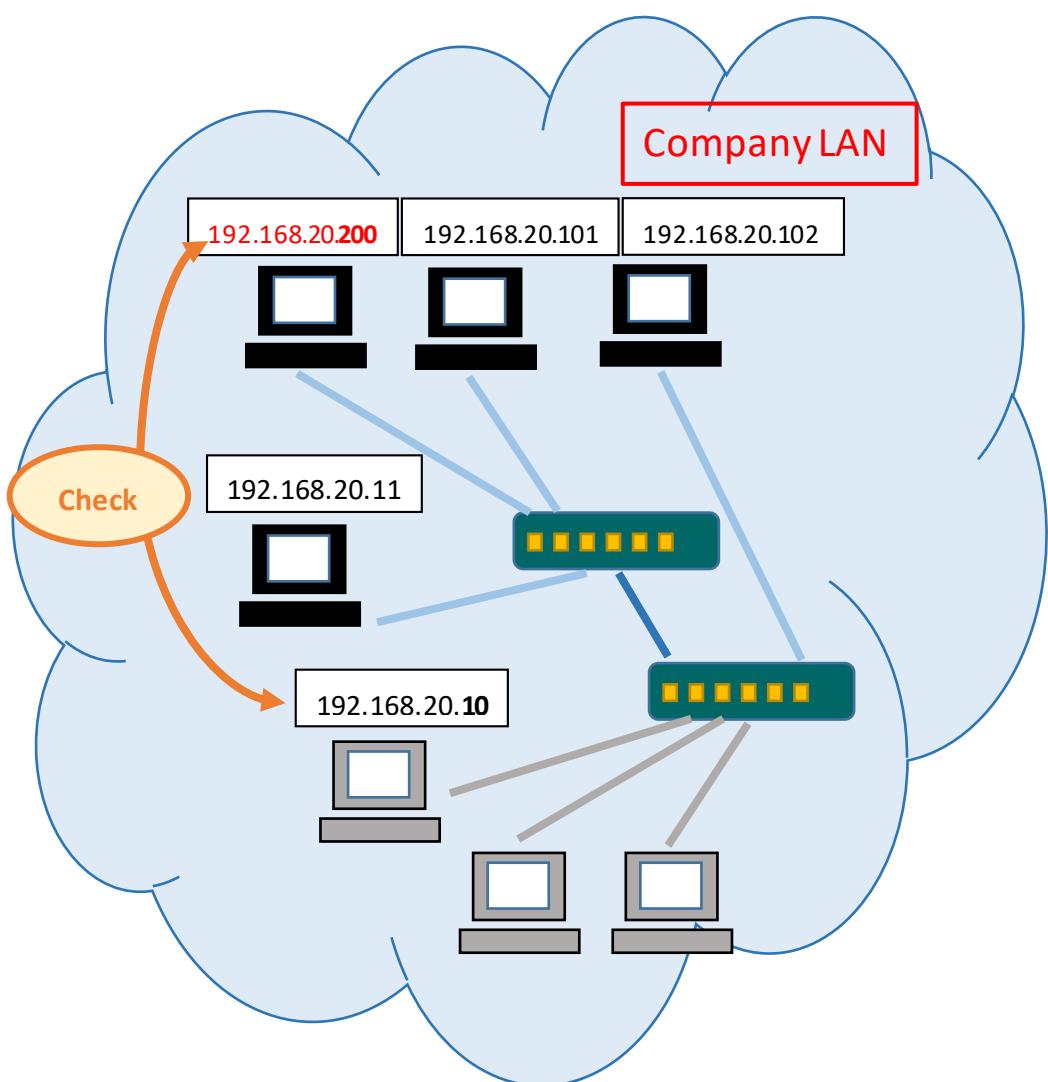
Option 1: Separate DeXipher Network

In the separate LAN setup, computers with DeXipher client installed are connected to the computer which has DeXipher Server configured and this network is kept separated from the remaining company's network. In this scenario a computer's IP address only need to be unique with respect to its own network, see scheme below:



Option 2: Integrated DeCipher Network

The DeCipher Network can also be integrated into the company's network. In this case it is important that the network architecture ensures that each computer has its own unique IP address, and no loop connection can be formed within the LAN:

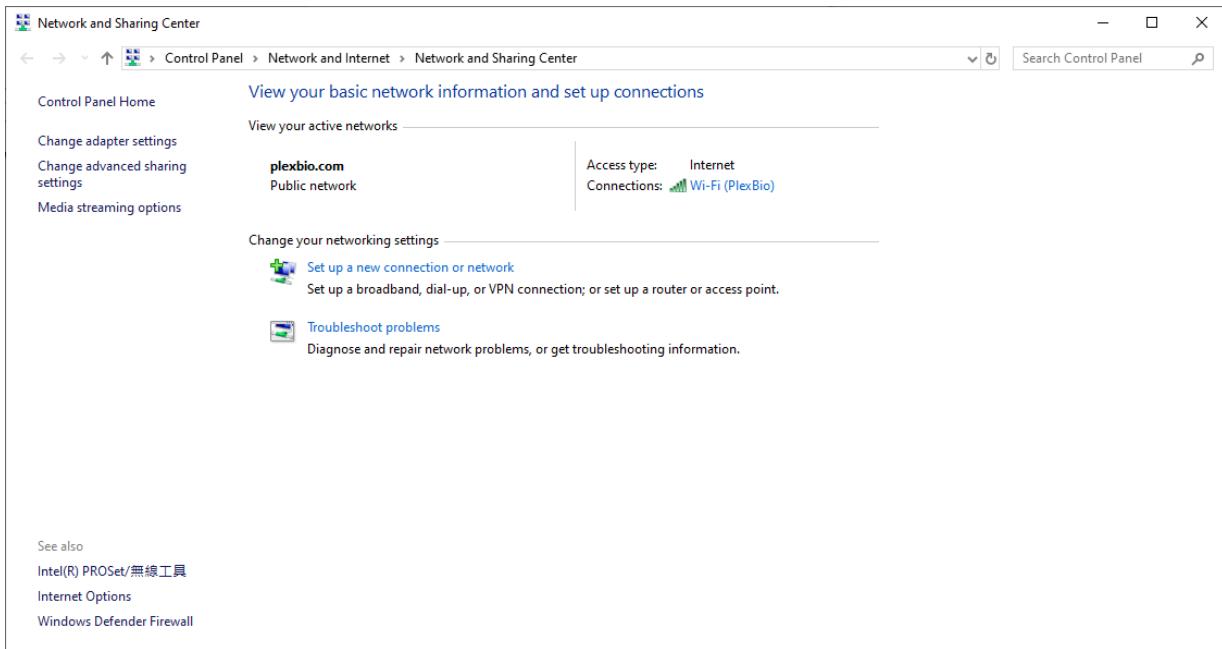


The DeCipher Network setup requires a static IP address for the computer hosting DeCipher Server. The static IP is required to facilitate the DeCipher client login process (see section 5.1.). DeCipher client computers do require a static IP address.

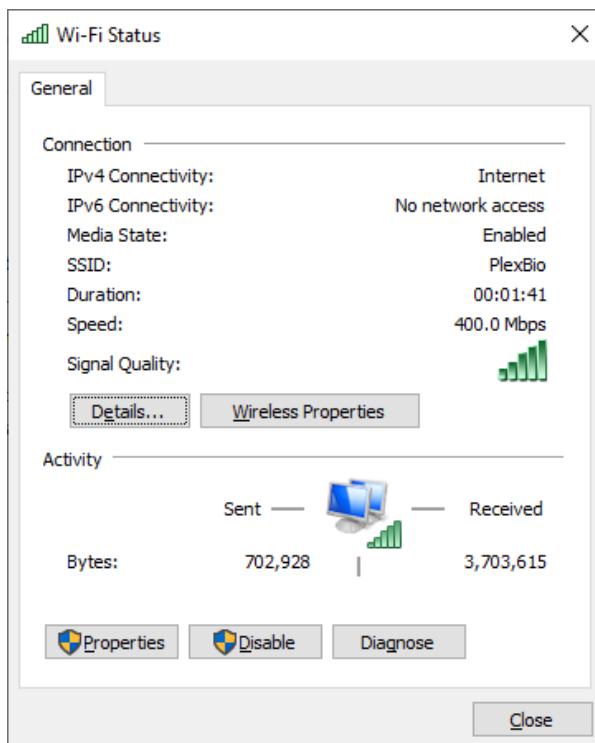
Static IP Address Setup.

To manually setup a computer's IP address, follow the steps below. Consult your network administrator or the DHCP server's user manual for more details on how to setup a static IP address within your network. The instruction below may not apply to your network architecture.

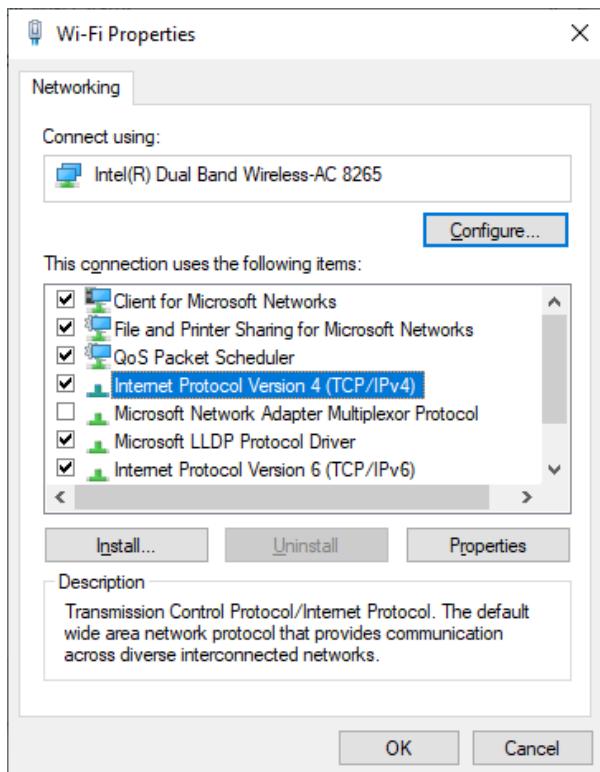
- (1) Open Control Panel → Network and Internet → Network and Sharing Center → and click on the connection (here: "Wi-fi (PlexBio)")



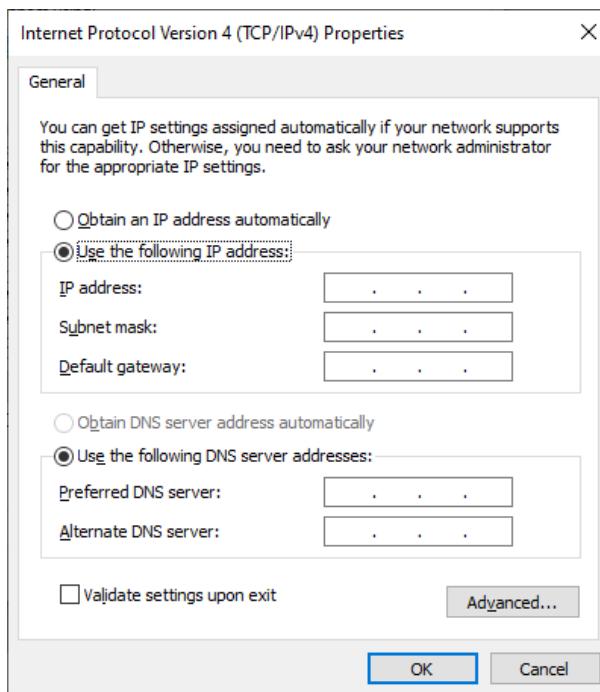
- (2) Click "Properties"



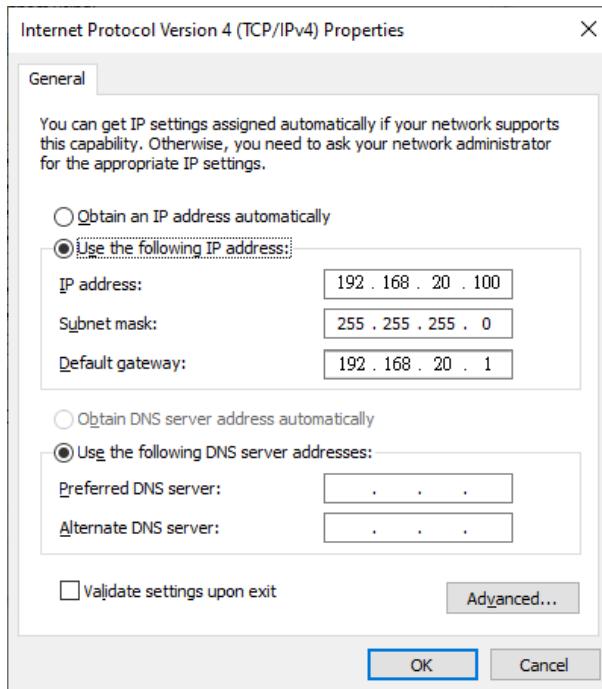
(3) Choose the item “Internet Protocol Version 4 (TCP/IPv4)”, then click “Properties”.



(4) Manually setup IP address and manually setup DNS.



- (5) Fill in IP address, Subnet mask, and Default gateway with the value of 192.168.0.100, 255.255.255.0, and 192.168.0.1, respectively.



- (6) Optional: Fill in “Preferred DNS Server” and “Alternate DNS Server”.

- (7) Click OK to save changes. Confirm changes by clicking “Details” in the status pop up.

Wi-Fi Status

General

Connection	Internet
IPv4 Connectivity:	Internet
IPv6 Connectivity:	No network access
Media State:	Enabled
SSID:	PlexBio
Duration:	00:01:41
Speed:	400.0 Mbps
Signal Quality:	<div style="text-align: right;">[Signal Strength Bar]</div>

[Details...](#) [Wireless Properties](#)

Activity

Sent — Received

Bytes: 702,928 | 3,703,615

[Properties](#) [Disable](#) [Diagnose](#)

Network Connection Details

Network Connection Details:

Property	Value
Connection-specific DN...	
Description	Intel(R) Dual Band Wireless-AC 8265
Physical Address	68-EC-C5-5B-ED-84
DHCP Enabled	No
IPv4 Address	192.168.20.100
IPv4 Subnet Mask	255.255.255.0
IPv4 Default Gateway	192.168.20.1
IPv4 DNS Server	
IPv4 WINS Server	
NetBIOS over Tcpip En...	Yes
Link-local IPv6 Address	fe80::dcd2:27ed:139a:5b4b%4
IPv6 Default Gateway	
IPv6 DNS Servers	fec0:0:0ffff::1%1 fec0:0:0ffff::2%1 fec0:0:0ffff::3%1

[Close](#)

What to do if the network is temporarily not working?

DeXipher client must be connected to DeXipher Server to operate. If the network is temporarily not available, but a time-sensitive analysis needs to be performed, it is possible to install a copy of DeXipher Server locally to operate DeXipher client in the standalone configuration (refer to section 4.2.1.). Follow instruction in section 5.1 on how to login to the standalone configuration (using Server IP 127.0.0.1). For first time login into the standalone configuration, login using the account information provided by PlexBio Co. Ltd or your local distributor. The accounts from the network configuration are not available in the standalone configuration.

Note: Using standalone configuration (section 4.2.1) while the network is not available allows to create, run and analyze assays. However, none of the information stored on the network server will be available in the standalone configuration and all information created in the standalone configuration will not be automatically synchronized with the network DeXipher Server.

All required ENC files and Calibration ENC files need to be imported into the standalone configuration. Protocols can be imported or newly created. All assay specific APPs already installed on the DeXipher client computer will be available in the standalone configuration and do not need to be installed again.

If required, all data generated using the standalone configuration must be manually transferred to the network server (see section 9.5). Note that only files that are confirmed (status *FINISHED*; see section 8.3) can be exported from the standalone configuration. If an IntelliPlex assay was analyzed while operating in the standalone configuration, the assay-lot-specific ENC file must be imported before the results can be confirmed and exported. To import results to the network DeXipher server, login to the network server through any DeXipher client and follow the import instruction in section 9.5. Keep in mind that all file related permissions will be included in the exported file. It is therefore important to ensure that standalone permissions are compatible with the DeXipher network configuration (section 9.5 and 9.6).

To use protocols from the network configuration in the standalone configuration, it is required to have a DeXipher client copy installed on the computer that hosts the DeXipher Server. Login to the DeXipher client on the DeXipher Server computer will allow access to all information stored in the DeXipher Server network database (refer to section 5.1 on how to login using Server IP 127.0.0.1) and protocols can be exported and manually imported to any standalone configuration.

4.2.3 Windows-related Settings and Firewall

Consult with your network administrator to ensure proper settings of your Firewall and Antivirus software. The Firewall settings on the DeXipher Server computer must allow the APP “Commons Daemon Service Runner” to communicate through the Firewall.

To allow this APP to communicate through Windows (Defender) Firewall, go to “Control Panel” → “System and Security” → “Windows (Defender) Firewall” → “Allow a program/app or feature through Windows Defender/ Firewall” → “Allow another app...” and browse to “C:\Program Files\Apache Software Foundation\Tomcat 9.0\bin\” to select “Tomcat9.exe”.

DeXipher network must further be able to connect to port 443 and 8080.

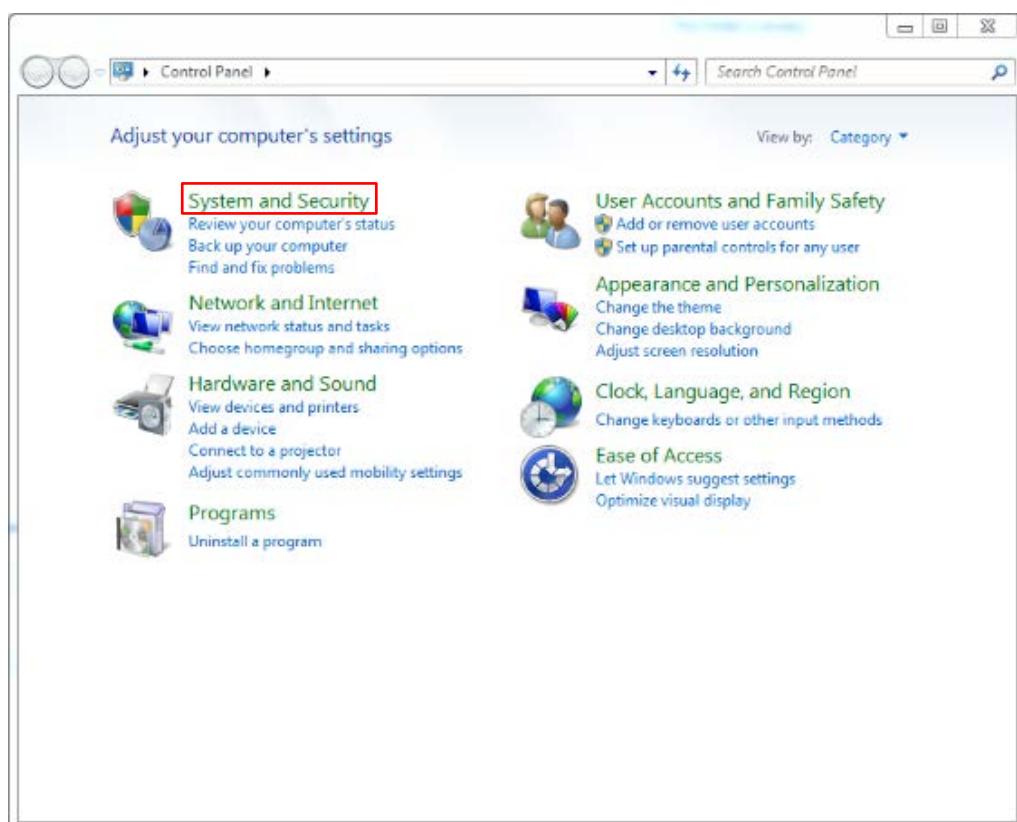
Windows-related Settings:

DeXipher requires certain Windows specific settings to function properly. If needed, follow the steps below to adjust /confirm Windows settings:

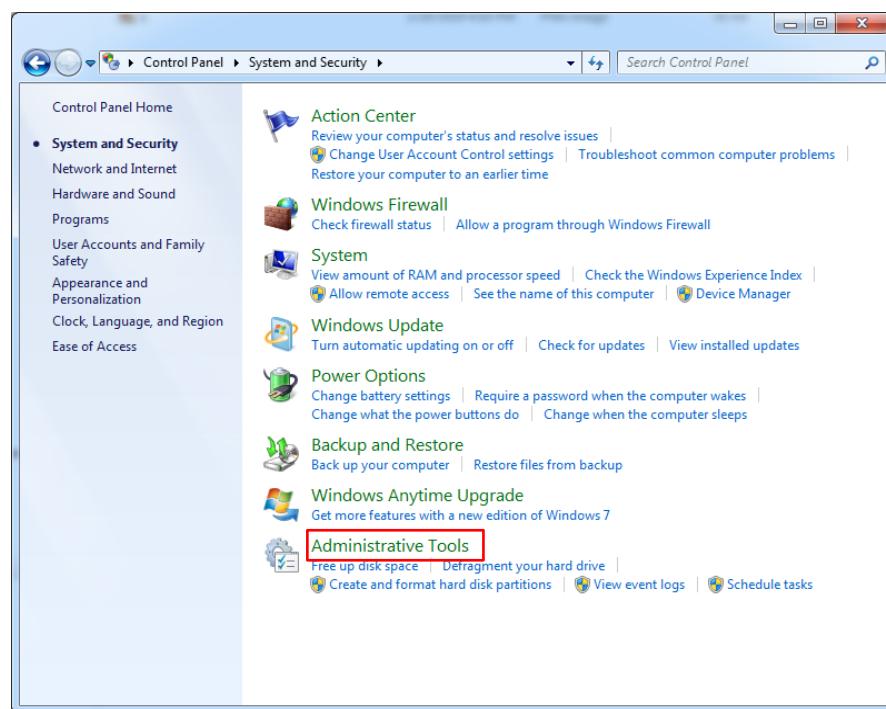
Windows 7/10:

Apache Tomcat:

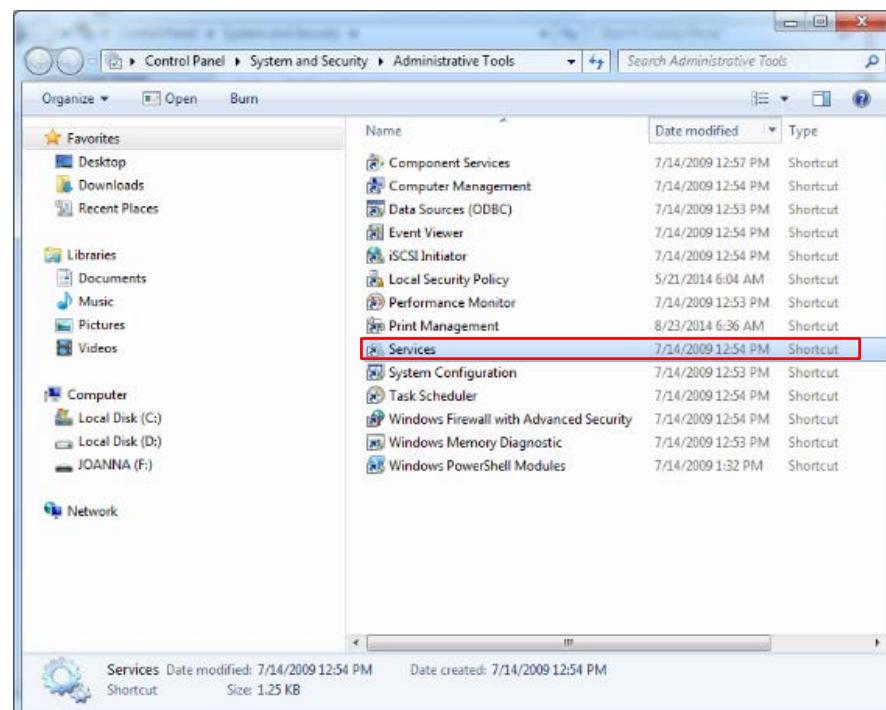
1. Go to Control Panel
2. Select System and Security



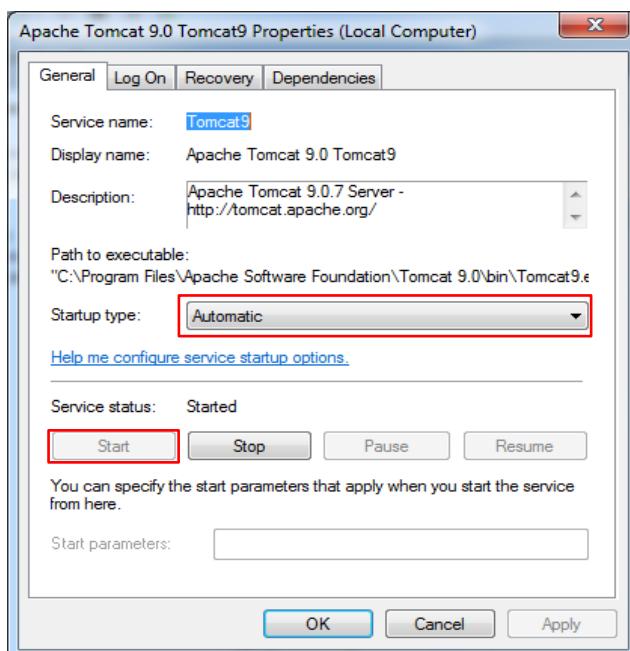
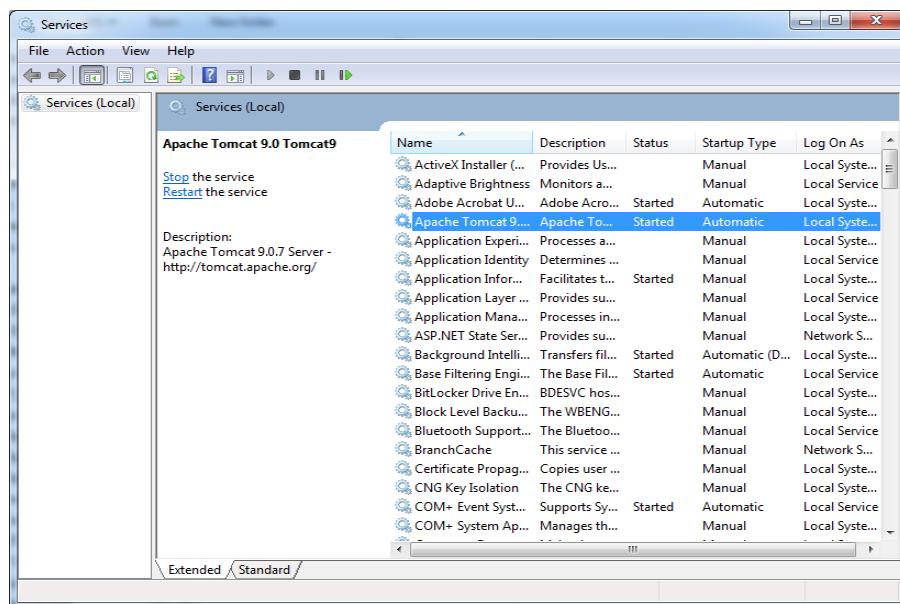
3. Select Administrative Tools



4. Select Services



5. If **Apache Tomcat** is not set to **Started** and **Automatic**, double click Apache Tomcat and set Startup type to Automatic and Service Status to Start; click Apply to save settings.

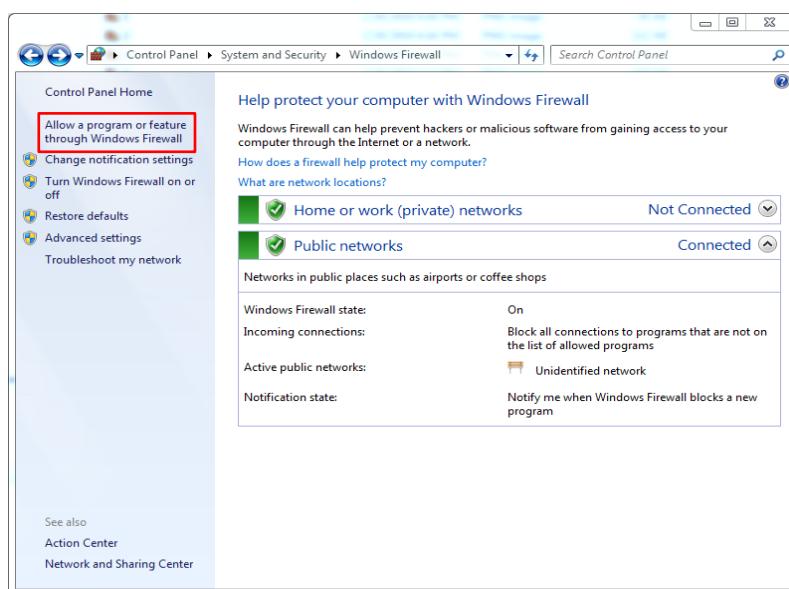


Firewall:

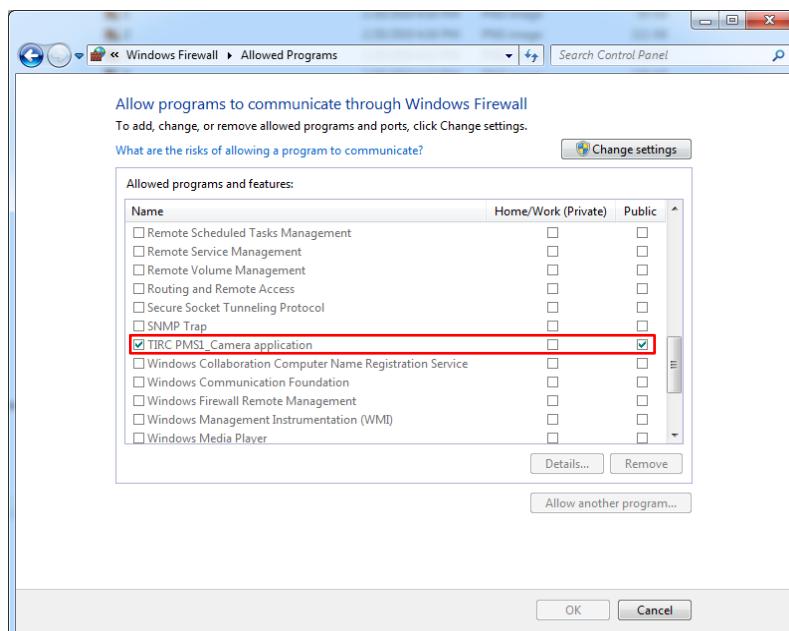
If features of the DeXipher software are blocked by a firewall, DeXipher may not run properly. Ensure that the firewall does not block DeXipher software.

Follow instruction below to setup Windows Firewall:

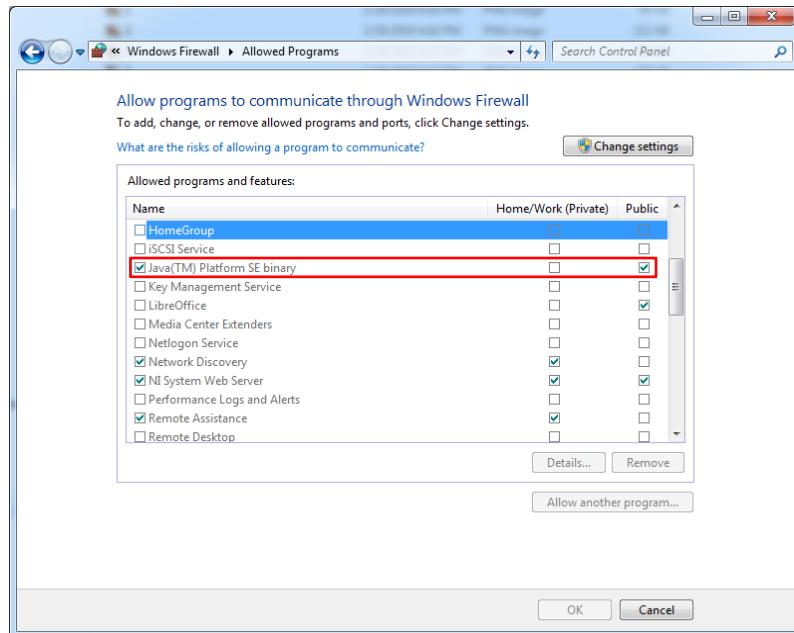
1. Go to **Control Panel**
2. Select **System and Security**
3. Select **Windows Defender/Firewall**
4. Select **Allow a program/app or feature through Windows Defender/Firewall**



5. Allows TIRC PMS1_Camera application



6. Allows everything that starts with **Java** (might be different from the picture)



7. Allows both Private & Public if there is WiFi connection

4.3 Uninstallation

To uninstall DeXipher, go to Windows “Control Panel/Programs” (Windows 7) or “Apps and Features” (Windows 10) and uninstall the following programs from PC: DeXipher, DeXipher_Server (if installed), AVT Vimba, and Apache Tomcat 9.0. If you have any issues with uninstallation, contact PlexBio Co., Ltd. or your local distributor for further help.

Chapter 5. Starting DeXipher™

5.1 Login

I. Before Starting DeXipher

Make sure all cables are connected properly. Refer to chapter 3.3 for further details.

Turn on PlexBio 100 Fluorescent Analyzer. The PlexBio 100 Fluorescent Analyzer needs approximately 15 minutes to warm up. The lid must be closed and remain closed until the warm-up process is completed*. The indicator light on the front will be steady pink when warming up and will turn white once the instrument is ready to use.

***Do not open the lid when the system is warming up.** Opening the lid will stop the initialization step. System restart is required to complete warm-up process after the lid has been closed again.

II. Starting DeXipher

Turn on the PC and wait until Windows has been fully loaded.

To start DeXipher, click on the application icon on your desktop (Fig. 8) or select DeXipher from the Windows Start menu.



Fig. 8. DeXipher icon

Note: Starting DeXipher for the first time may trigger a response from Windows Firewall (or any other installed Firewall). It is important to give permission to all application.

III. DeXipher Login

The default login setting for DeXipher client is for the standalone configuration (127.0.0.1 in the “Server list box”, see Fig. 9). If the DeXipher client was installed as part of a network, the DeXipher server IP address needs to be added into the “Server list box”. Also ensure that the DeXipher server is available. DeXipher client will not function without active connection to DeXipher server.

IMPORTANT: When starting DeXipher client, the last used IP address will appear in the “Server

list box". The IP address can be changed before login. Always confirm that the IP address is correct. Note that it may take up to several minutes to connect to a server or to report any server connection errors in case the server is not available using the specified IP address. Systems that have no access to DeXipher Server within a network must have DeXipher Server installed locally and use "127.0.0.1".

IV. DeXipher First-time Login

For first time login, login using the account information provided by PlexBio Co. Ltd or your local distributor. Confirm correct **Server IP** in the "Server list box" (Standalone: 127.0.0.1) and enter the provided **Login ID** and **Password**, then click **Login**.

Changing the password after the first login is highly recommend. Creating additional Account(s) is optional. For an overview of account types and their features and how-to setup Accounts in DeXipher, refer to section 12.2. New accounts can login using their respective **Login ID** and **Password** information.



Fig. 9. Login screen

5.2 DeXipher User Interface

After logging in, the DeXipher User Interface as shown in Fig. 10 will appear.

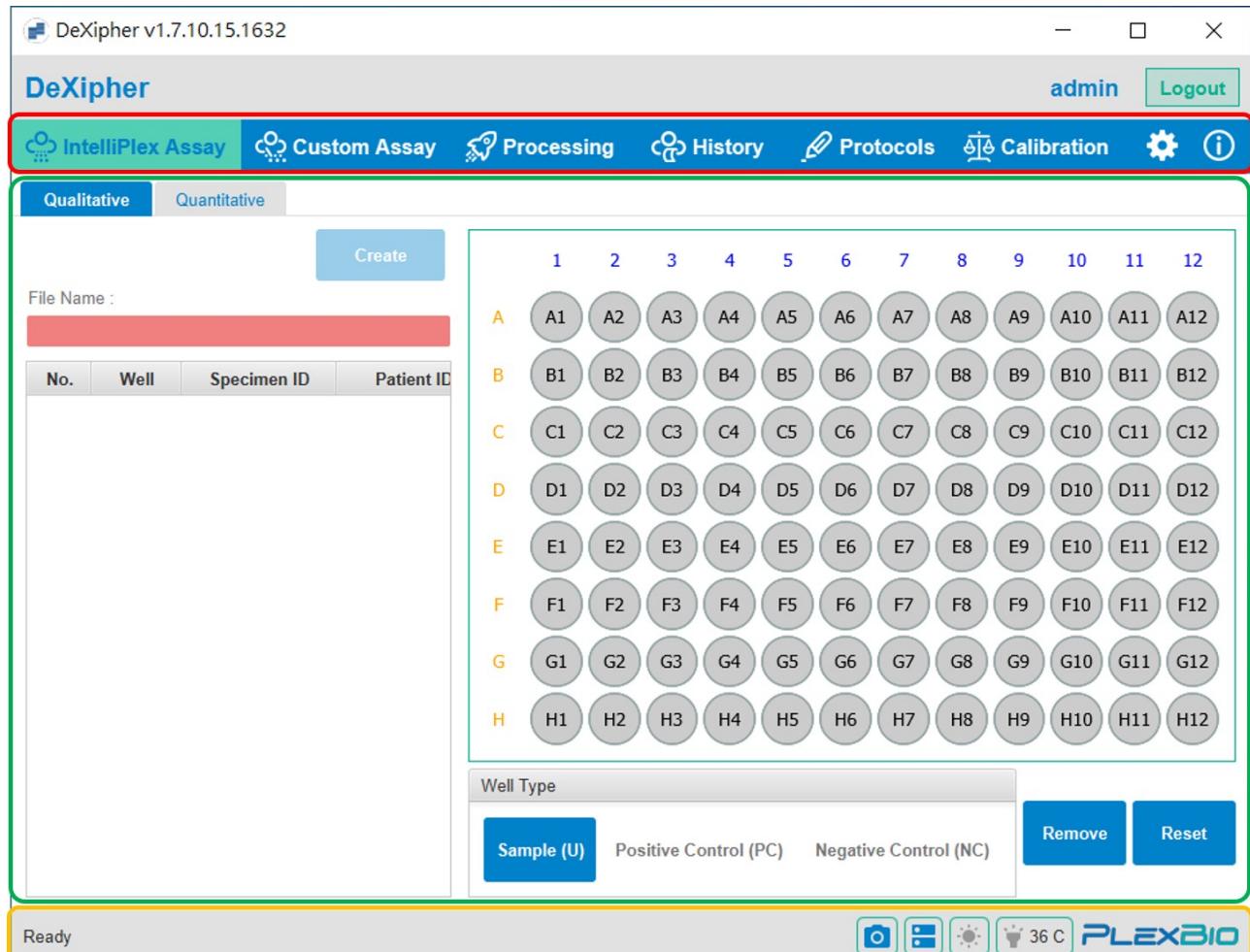


Fig. 10. DeXipher homepage

The ID of the actively logged in user is displayed on the upper right, next to the Logout button. In Fig. 10, User “admin” is logged in. The Logout button can be used to log the active account out from the system to allow a new login. DeXipher client supports only one account being actively logged in.

Different functions of DeXipher can be accessed by clicking on the respective tab displayed on the top of DeXipher User Interface (Fig. 10, red box). Note that a slightly different set of tabs will be displayed on the User Interface of DeXipher Basic or RUO.

The main section of the DeXipher User Interface shows the function available in the selected tab. In Fig. 10, the **IntelliPlex Assay tab** is shown (green box). Refer to the chapters listed in Table 6 for more details on different functions featured in each tab.

List of tabs/functions available in DeXipher:

Tab/Function (License Type)	Brief Description	Reference
IntelliPlex Assay (RUO)	The IntelliPlex Assay tab serves as interface to setup the analysis for IntelliPlex-branded Assays. Well selection and sample information are added during setup.	Chapter 6
Custom Assay (RUO)	The Custom Assay tab allows to perform Quick Read (RUO/Basic) or to analyze assays using Quantitative or Qualitative protocols (RUO only). Well selection and sample information are added during setup.	Chapter 7
Processing (RUO/Basic)	The Processing tab allows the user to follow the process while wells are being analyzed, and results can be accessed. All well pictures can be visualized. Sample information can be edited. Assays can be confirmed.	Chapter 8
History (RUO/Basic)	The History tab allows access to saved assays and reports. Different protocols can be applied to results. Results can be exported.	Chapter 9
Protocol (RUO)	The Protocol tab supports the setup of custom-made protocols for quantitative or qualitative assays.	Chapter 10
Calibration (RUO/Basic)	The Calibration tab supports the monthly calibration process of the PlexBio™ 100 Fluorescent Analyzer.	Chapter 11
Settings (RUO/Basic)	The Settings tab allows to change DeXipher settings, including User management. The settings differ between different license types of DeXipher.	Chapter 12
Information (RUO/Basic)	The Information tab provides software specific information such as license type, software version number and log file.	Chapter 13

Table 6. Overview of tabs/functions in DeXipher

The bottom area of the DeXipher homepage (Fig. 10, orange box) displays the operational and current hardware status of the PlexBio 100 Fluorescent Analyzer. Once the connection between the PlexBio 100 Fluorescent Analyzer and the DeXipher software is established, the left area will show the current operational status.

Status Text	Operational Status	Description
Ready	Idle	PlexBio 100 Fluorescent Analyzer is ready to use but idle.

Calibrating	Calibration	Calibration of PlexBio 100 Fluorescent Analyzer is performed.
Goto well ##	Reading wells	Plate-arm is moving to reading position of the indicated well ##.
Recognizing ## (#/21)	Reading wells	PlexBio 100 Fluorescent Analyzer is analyzing indicated frame # of the specified well ##.
Motor homing, please wait	Reading wells; Calibration	Plate-arm is moved to its home position.
Error code	When a system error occurred	Error code shows up when unexpected error occurs

Table 7. Overview of operational status

Note: If no status is shown, the connection between the PlexBio 100 Fluorescent Analyzer and the DeXipher software has not been established. Ensure all cables are connected as described in section 3.3, and the PlexBio 100 Fluorescent Analyzer is plugged in and switched on.

The current hardware status of the PlexBio 100 Fluorescent Analyzer is shown on the right side of the bottom area (Fig. 10, orange box). The status icons are shown enlarged in Fig. 11 and are in the following order:

- PlexBio 100 camera connection status
- PlexBio 100 host connection status
- White light usage status
- Fluorescent light usage status and current temperature of fluorescent light



Fig. 11. System status bar

The PlexBio 100 Fluorescent Analyzer is ready for analyzing once the status of all icons except “White light usage status” have switched from grey to blue.

Any status not in blue after PlexBio 100 Fluorescent Analyzer is warmed up might indicate an error. The only exception is “White light usage status”; this status will change between grey and blue while the PlexBio 100 Fluorescent Analyzer is actively analyzing wells.

Note: The system will automatically shut down if the system temperature is over 70°C. If temperature status indicates error, restart the PlexBio 100 system, and if the problem persists, contact PlexBio Co., Ltd. or your local distributor.

Chapter 6. IntelliPlex Assay

The **IntelliPlex Assay tab** is only available in the RUO license of DeXipher. For DeXipher Basic, refer to section 7.1 for instruction on how to analyze wells.

The **IntelliPlex Assay tab** (Fig. 12) serves as interface to analyze all IntelliPlex-branded assays. The minimum requirement to setup an assay in the **IntelliPlex Assay tab** is selection of wells to be analyzed, specification of sample type in each well, and providing a unique name for the run (“File Name”). Additional sample information can be added.

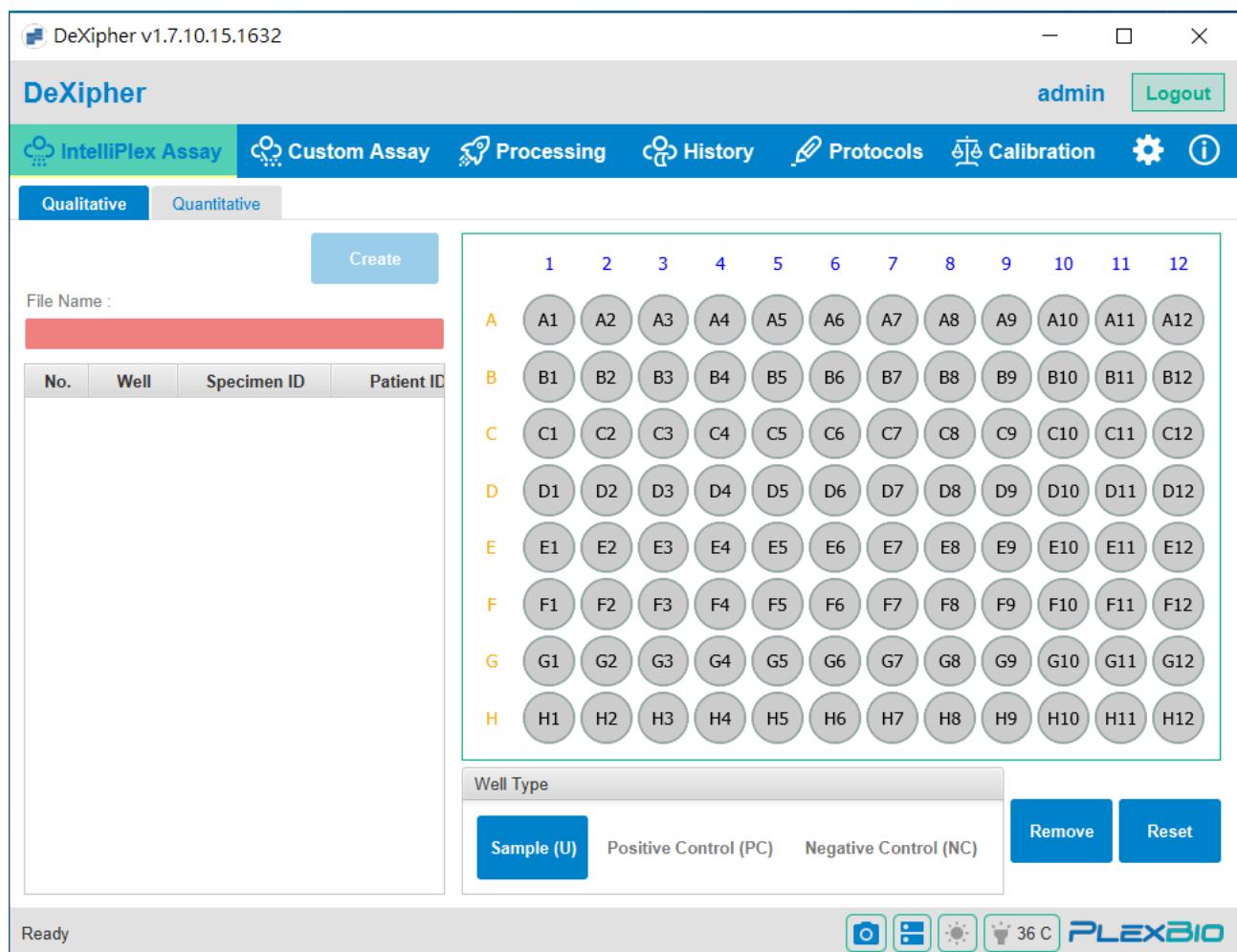


Fig. 12. IntelliPlex Assay Menu

DeXipher will automatically identify the IntelliPlex-branded assay kit (e.g. IntelliPlex EGFR Mutation Kit(RUO)) and lot number based on classified πCode image patterns unique for each IntelliPlex-branded assay kit and lot.

Results can be accessed as raw data and exported as XLSX file format. The exported XLSX file contains all identified πCode MicroDiscs and their respective measured fluorescent intensity as well as the median fluorescent intensity of πCode MicroDiscs grouped by identity. In addition, DeXipher will also generate a report for the results for research use only (RUO) purposes.

The user can select between Qualitative and Quantitative sub-tab within the **IntelliPlex Assay tab**. The selection depends on which IntelliPlex-branded assay is being analyzed. Refer to the IFU of the IntelliPlex-branded assay.

The PlexBio 100 Fluorescent Analyzer needs monthly calibration. Refer to chapter 11 for more details on how to calibrate PlexBio 100 Fluorescent Analyzer.

Important: Before running an IntelliPlex-branded assay on DeXipher, ensure that the assay-specific APP is installed and that the ENC file corresponding to the assay's lot number is imported. See section 6.1 and section 12.4 for more details.

Note: DeXipher Basic will not support analyzing IntelliPlex-branded assays. Contact PlexBio Co., Ltd. or your local distributors for DeXipher RUO license type.

6.1 Install IntelliPlex Assay APP

Before using an IntelliPlex-branded assay for the first time, users should install the assay specific APP for the DeXipher client software. The APP is required to generate reports. If a laboratory uses multiple DeXipher clients, the APP must be installed on each system even if connected to the same DeXipher Server. Note that APPs are only supported by DeXipher RUO; DeXipher Basic does not support APPs.

Follow the instruction below to install IntelliPlex-branded assay APP:

- Download the APP specific for the IntelliPlex-branded assay from www.plexbio.com to a folder on the computer with DeXipher client installed. In case of questions, contact PlexBio Co., Ltd. or your local distributor.
- Unpack the downloaded file (a software that can extract RAR files is required)
- Double click “Installer.exe” in the unpacked folder to start the installation.
- The APP Installer window will open. Already installed components will be indicated in the window. Click “Install” to continue:
 1. Allow all component installation and upgrades.
 2. Accept all terms in the license agreements.
 3. Several pop-up and installation windows may open during installation. Click “OK” or “Next” as appropriate to continue installation.
 4. Installation may take several minutes to complete.
- Acknowledge successful installation of the APP in the InstallShield window.
- The APP Installer window can now be closed.
- Start DeXipher and login with your Username and Password. The APP is now available to you.

Note: PlexBio Co. Ltd. may issue APP updates. Updated APPs will be compatible with older lots of the IntelliPlex-branded assay, but new lots may no longer work with an outdated APP. APP updates should therefore be installed immediately once issued.

Note that results derived from analyzing IntelliPlex-branded assay with the PlexBio 100 Fluorescent Analyzer using a DeXipher client without the corresponding APP installed (or an outdated version of the APP) are saved. To generate reports and access the IntelliPlex-branded assay results, the latest APP must be installed. It is recommended to install the APP before analyzing wells with the PlexBio 100 Fluorescent Analyzer.

Uninstall APP:

To uninstall an APP, go to Windows “Control Panel/Programs” (Windows 7) or “Apps and Features” (Windows 10) and uninstall the desired APP. If you have any issues with uninstallation, contact PlexBio Co., Ltd. or your local distributors for further help.

6.2 Reading an IntelliPlex Assay

Before using an IntelliPlex-branded assay, it is recommended to have the assay-specific APP installed and the lot-specific ENC file imported. DeXipher will analyze wells and save results but not generate a report if the IntelliPlex-branded assay APP or the lot-specific ENC file is missing. Refer to section 6.1 for instruction on how to install an APP and section 12.4 on how to import an ENC file. Refer to chapter 8 on how to generate a report and chapter 9 on how to access reports.

The PlexBio 100 Fluorescent Analyzer needs monthly calibration. Refer to chapter 11 for more details on how to calibrate PlexBio™ 100 Fluorescent Analyzer.

The Instruction for Use (IFU) of the IntelliPlex-branded assay will provide information on the type of assay: Qualitative (section 6.2.1) or Quantitative (section 6.2.2). Proceed to the respective chapter.

6.2.1 IntelliPlex Assay - Qualitative

This **IntelliPlex Assay Kit** function is for IntelliPlex-branded assays that provide a **qualitative** result. To analyze a qualitative IntelliPlex branded assays, the following information needs to be entered:

File Name:

Provide a unique name for the analysis.

If there is no name or the name has already been used, the “Create” button will remain disabled.

Well Selection:

Select the type of sample and the position in the 96-well plate by selecting the “Well Type” (U = Sample (Unknown), PC = Positive Control, NC = Negative Control) and then the position in the schematic display of a 96-well plate.

To generate a valid report in DeXipher, each test requires the inclusion of a PC (Positive Control, selected wells are marked in red) and a NC (Negative Control, selected wells are marked in dark grey). Wells selected for samples are marked in yellow. To select multiple sample wells, keep the left mouse button pressed while moving over wells to select.

Note: Load 96-well plates lane-wise. The IntelliPlex-technology processes 96-well plates by lanes, which are defined by the 8 well strip going from A to H. A 96-well plate consists of 12 lanes (1-12); each lane consists of 8 wells (A-H).

Remove:

To remove a well selection, mark each well that needs to be cleared (marked wells will be highlighted with a red box) and click Remove.

Reset:

To reset the whole plate (remove all entries), click Reset.

Sample Information:

Provide information such as Specimen ID and Patient ID for each well containing a sample. Providing information in the IntelliPlex Assay tab is optional. The information can also be added in the Processing tab before, during or after the read. Sample information can be added/ edited until the file is confirmed, and the status is set to FINISHED (chapter 8).

Create:

After entering a valid File Name and selecting wells, the “Create” button will be active. Click on it to save the entered information and to proceed to **Processing tab** to read the plate.

All information has been saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can still be edited or deleted before it is read.

Note: To analyze multiple IntelliPlex-branded assays or different lots of the same assay on the same plate in the same run, simply select the position of all PC and NC pairs on the plate (see example below). Select all wells containing sample. DeXipher will automatically associate the PC, NC and samples with their respective assays and lots. Make sure that for each assay-lot only one PC and one NC is selected. If multiple PC or NC controls are associated with the same lot of an assay, the report will not be available.

Editing a file:

Any created file that has not been analyzed can be edited by clicking “Edit/Back” in the **Processing tab** (chapter 8). DeXipher will return to the **IntelliPlex Assay tab** and allow updating the well selection, well type and File Name. To confirm or discard changes, the following options are available:

- **Update:**

To save modification and update the original created file and proceed to the **Processing tab**, click “Update”.

- **New:**

To abort editing and discard all changes, click “New”. The content of the original created file will not be modified and can be accessed in the **History tab**.

- **Delete:**

To delete a created file permanently, click “Delete”. The file and all information associated with it will be deleted and can no longer be received in the **History tab**.

See the example below (Fig. 13) on how to setup IntelliPlex-branded assay(s) using the **IntelliPlex Assay - Qualitative tab**:

DeXipher v1.7.10.15.1632

admin Logout

Qualitative Quantitative Step 5

Create

File Name : Step 1
IntelliPlex Kras assay

No.	Well	Specimen ID	Patient ID
U1	C1	PB002	NM3z
U2	D1		Step 4
U3	E1		
U4	F1		
U5	G1		
U6	H1		
U7	A2		
U8	B2		
U9	C2		

Step 2 1 2 3 4 5 6 7 8 9 10 11 12

Step 3 A B C D E F G H

Well Type

Sample (U) Positive Control (PC) Negative Control (NC) Remove Reset

Ready

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Fig. 13. IntelliPlex Assay - Qualitative

If only one IntelliPlex-branded assay is analyzed on the 96-well plate:

- Step 1. Enter **File Name** (Example: "IntelliPlex Kras assay(RUO)").
- Step 2. Select **POS** Control (PC1; well A1; red) and **NEG** Control (NC1; well B1; dark grey).
- Step 3. Select **Sample** wells (Example: U1-9 in wells C1 to H1 and A2 to C2; yellow wells).
- Step 4. Optional: Enter sample descriptions. The plate format is available as reference.
- Step 5. Click "Create" to proceed to the **Processing** function (go to step 14 below)

Note: All information has been saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can be edited or deleted before reading.

If more than one IntelliPlex-branded assay is analyzed on the 96-well plate or if multiple lots of the same IntelliPlex-branded assay are present on the same 96-well plate (Fig. 14):

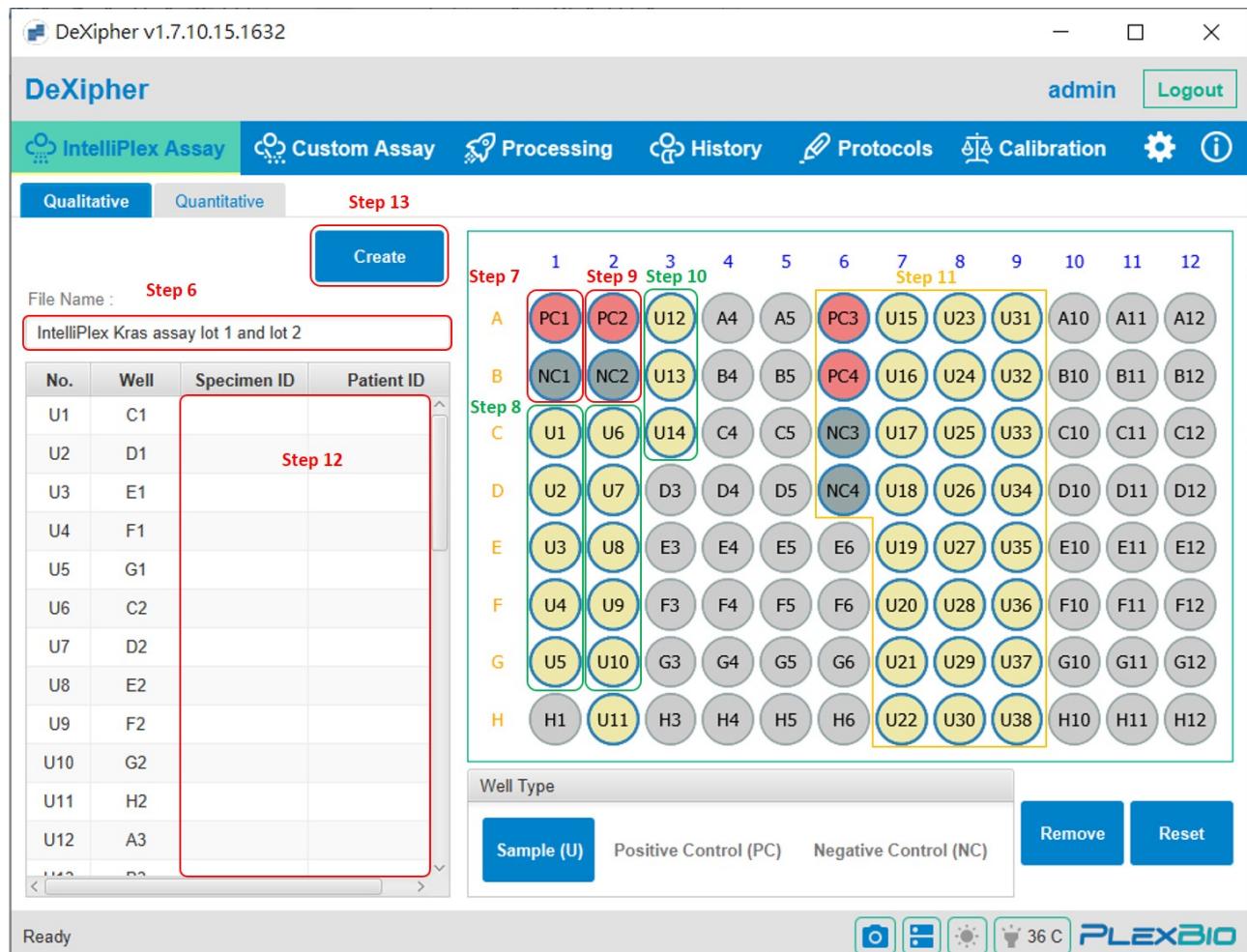


Fig. 14. IntelliPlex Assay - Qualitative: multiple assays/ lots

- Step 6. Enter **File Name** (Example: “IntelliPlex Kras assay(RUO) lot 1 and lot 2”).
- Step 7. Select **POS** Control (PC1; well A1; red) and **NEG** Control (NC1; well B1; grey) associated with the first IntelliPlex-branded assays.
- Step 8. Select **Sample** wells associated with the first IntelliPlex-branded assays (wells C1 to G1; sample U1 to U5; yellow wells).
- Step 9. Select **POS** Control (PC2; well A2; red) and **NEG** Control (NC2; well B2; grey) associated with the second IntelliPlex-branded assays or different lot of same assay.
- Step 10. Select **Sample** wells associated with the second IntelliPlex-branded assays or a different lot of the same IntelliPlex-branded assays (wells C2 to H2 and A3 to C3; sample U6 to U14; yellow wells)
- Step 11. Repeat step 9 and 10 if additional assays or different lots of the same assay are run in parallel on the same 96-well plate. In the shown example, two more assays are selected.

PC and NC for assay 3 are in wells A6 (PC3) and C6 (NC3); PC and NC for assay 4 are in wells B6 (PC4) and D6 (NC4). Samples are in wells A7 to H7, A8 to H8 and A9 to H9. DeXipher will automatically associate all samples to with their respective PC and NC controls based on assay and lot-specific πCode™ MicroDisc.

- Step 12. Optional: Enter sample descriptions. The plate format is available as reference.
- Step 13. Click “Create” to proceed to the **Processing tab** (go to step 14 below)

Note: All information has been saved to the File Name and can be retrieved in the **History** tab to read the assay at any time. The file can be edited or deleted before reading.

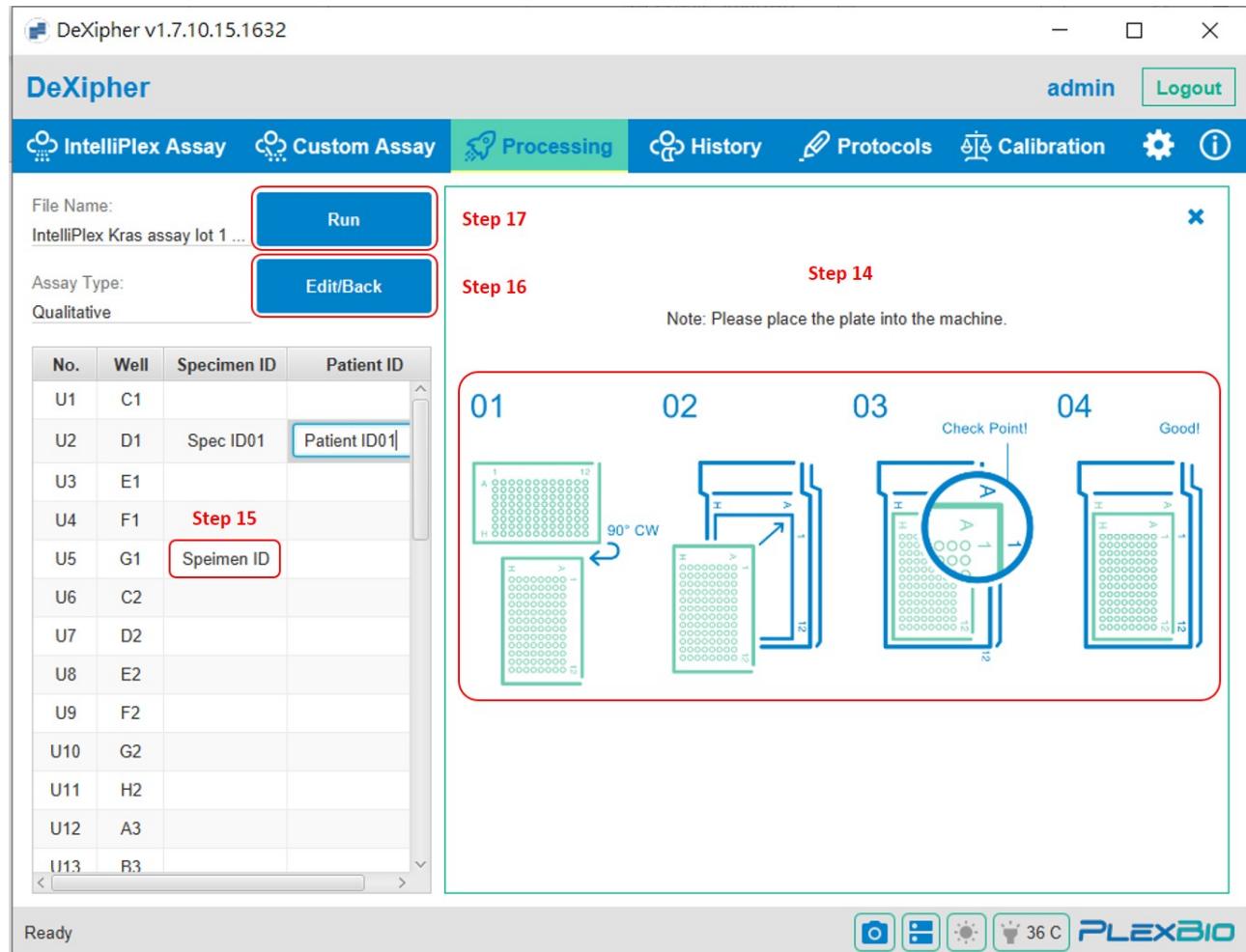


Fig. 15. IntelliPlex Assay - Processing

- Step 14. Follow the drawing in Fig. 15 to put the 96-well plate onto the stage (plate holder) with the correct orientation.
- Step 15. Specimen information can be entered prior, during or after the reading. To see the plate with sample selection, remove the plate instruction by clicking the “X” on the top right corner.
- Step 16. Click the “Edit/Back” button to change sample/control well selection in the 96-well plate.
- Step 17. Click “Run” to start reading.

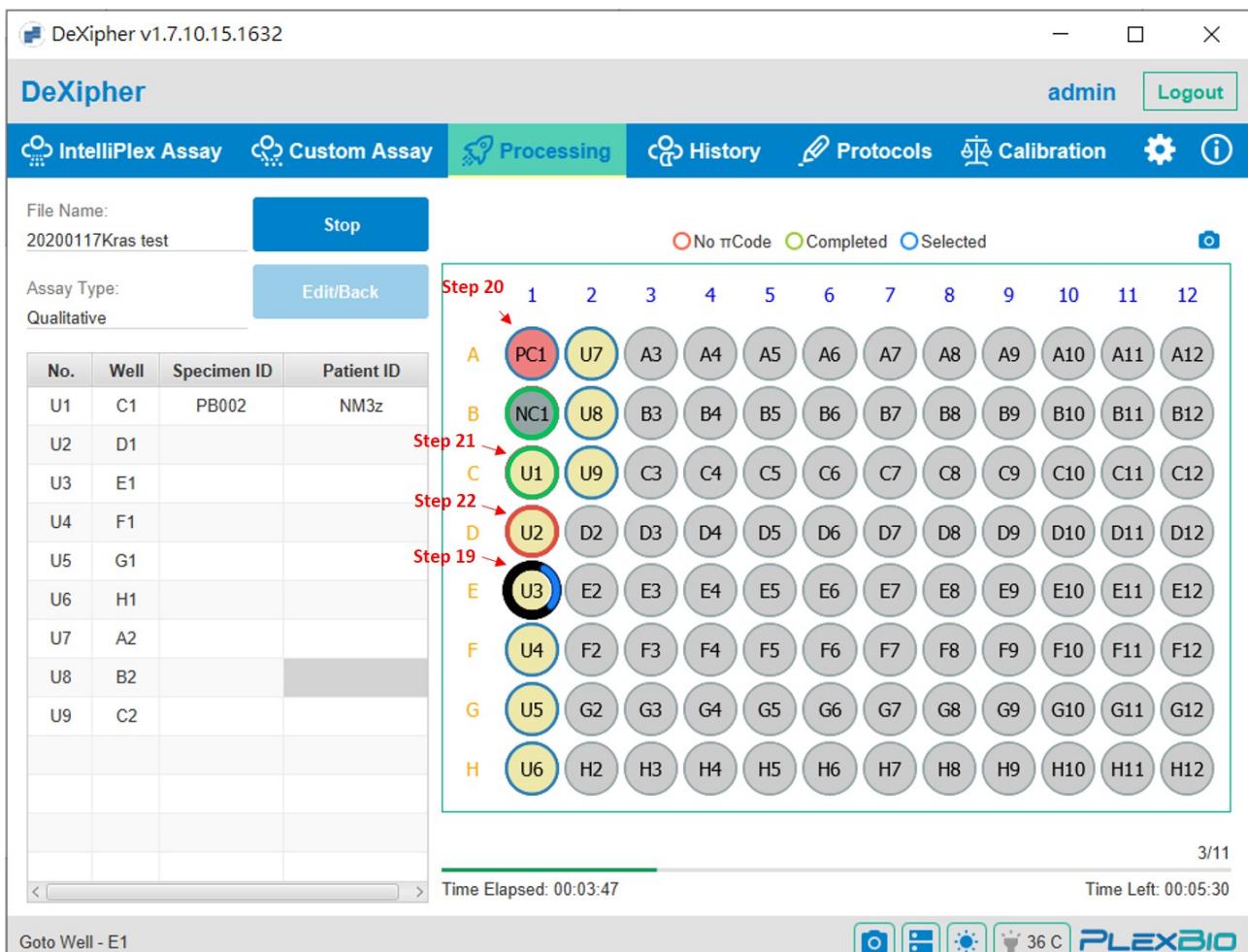


Fig. 16. IntelliPlex Assay - Processing - Run Status

- Step 18. PlexBio 100 Fluorescent Analyzer starts to read selected wells (Fig. 16).
- Step 19. Wells that are being read are circled black with a blue area moving around the circle (see well C2; it takes approximately 50 sec to read one well). The camera symbol on the top right corner of the 96-well plate allows to see a live image.
- Step 20. Wells that have not been analyzed are circled blue (=selected).
- Step 21. Wells that have been analyzed are circled green (=complete, see well H1) if πCode were recognized
- Step 22. Wells that have been analyzed are circled red (=No πCode, see well D1) if DeXipher was unable to recognize πCode in the well.

Note: Do not open the Lid while the PlexBio 100 Fluorescent Analyzer is analyzing wells. Opening the lid will cause a hard abort and terminate the analysis. The analysis cannot be resumed, and the results of unread wells will not be available. If – for any reason – a read needs to be stopped before it is completed, click **Stop** instead of opening the lid to terminate the analysis. The analysis still cannot be resumed, and the results of unread wells will not be available, but PlexBio 100 Fluorescent Analyzer will move the stage to its default position, where the 96-well plate can be taken out. Wait

until DeXipher confirms that the run has been successfully terminated before opening the lid.

- Step 23. Once DeXipher™ has analyzed all wells, a pop-up window will confirm that the read is completed.
- Step 24. Sample information can be added until the file has been confirmed and the status is *FINISHED*. Note: Do not confirm a file if the assay-lot-specific ENC file is not imported (Refer to chapter 8 for more information on how to confirm and change status to *FINISHED*).
- Step 25. All results are saved to the file and can be accessed at any time in the **History tab** (chapter 9). For each confirmed file, a report of the result will also be available.

6.2.2 IntelliPlex Assay - Quantitative

This **IntelliPlex Assay** function is for IntelliPlex-branded assays that provide a **quantitative** result. To analyze a quantitative IntelliPlex branded assays, the following information needs to be entered:

File Name:

Provide a unique name for the analysis.

If there is no name or the name has already been used, the “Create” button will remain disabled.

Well Selection:

Select the type of sample and the position in the 96-well plate by selecting the “Well Type” (U = Sample (Unknown), S = Standard, C = Control) and then the position in the schematic display of a 96-well plate.

To generate a valid report in DeXipher, each test requires the inclusion of a series of standards (selected wells marked in purple; refer to IFU of the IntelliPlex-branded assays for more details on the standards) and control(s) (selected wells are marked in red; refer to IFU of the IntelliPlex-branded assays for more details). Wells selected for samples are marked in yellow.

To select the sample, standard or control in duplicate (or higher replication), select the appropriate number from the **Sample Replicate** box. The replicate number for each sample can differ (e.g. duplicate reading for sample 1, single read for sample 2 etc.), but the replicate number for all standards and controls must be the same (e.g. all standards read as duplicates, all controls read as triplicates).

To select multiple wells at once, select multiple wells while keeping the left mouse button clicked. Samples will be filled lane-wise (A-H) as default. For example, if 2 replicates for samples are selected, selecting all wells in lane 1 and 2 will associate sample 1 to well A1 and B1, sample 2 to wells C1 and D1 all the way to sample 8 in wells G2 and H2. To change the direction of automatic well assignment, refer to section 12.1.

Note: Load 96-well plates lane-wise. The IntelliPlex-technology processes 96-well plates by lanes, which are defined by the 8 well strip going from A to H. A 96-well plate consists of 12 (1-12) lanes;

each lane consists of 8 wells (A-H).

Remove:

To remove a well selection, mark each well that needs to be cleared (marked wells will be highlighted with a red box) and click Remove.

Reset:

To reset the whole plate (remove all entries), click Reset.

Sample Information:

Provide information such as Specimen ID and Patient ID and Dilution Factor for each sample containing well. **The Dilution Factor is set to “1” by default and needs to be adjusted accordingly for each sample.** Providing this information in the **IntelliPlex Assay tab** is optional. The information can also be added in the Processing tab before, during or after the read. Sample information can be added/ edited until the file is confirmed, and the status is set to FINISHED (chapter 8).

Create:

After entering a valid File Name and selecting wells, the “Create” button will be activated. Click “Create” to save the entered information and to proceed to Processing tab to read the plate.

All information will be saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can be edited or deleted before reading.

Editing a file:

Any created file that has not been analyzed can be edited by clicking “Edit/Back” in the **Processing tab** (chapter 8). DeXipher will return to the **IntelliPlex Assay tab** and allow updating the well selection, well type and File Name. To confirm or discard changes, the following options are available:

• **Update:**

To save modification and update the original created file and proceed to the **Processing tab**, click “Update”.

• **New:**

To abort editing and discard all changes, click “New”. The content of the original created file will not be modified and can be accessed in the **History tab**.

• **Delete:**

To delete a created file permanently, click “Delete”. The file and all information associated with it will be deleted and can no longer be received in the **History tab**.

See the example (Fig. 17) on how to use the **IntelliPlex Assay - Quantitative**:

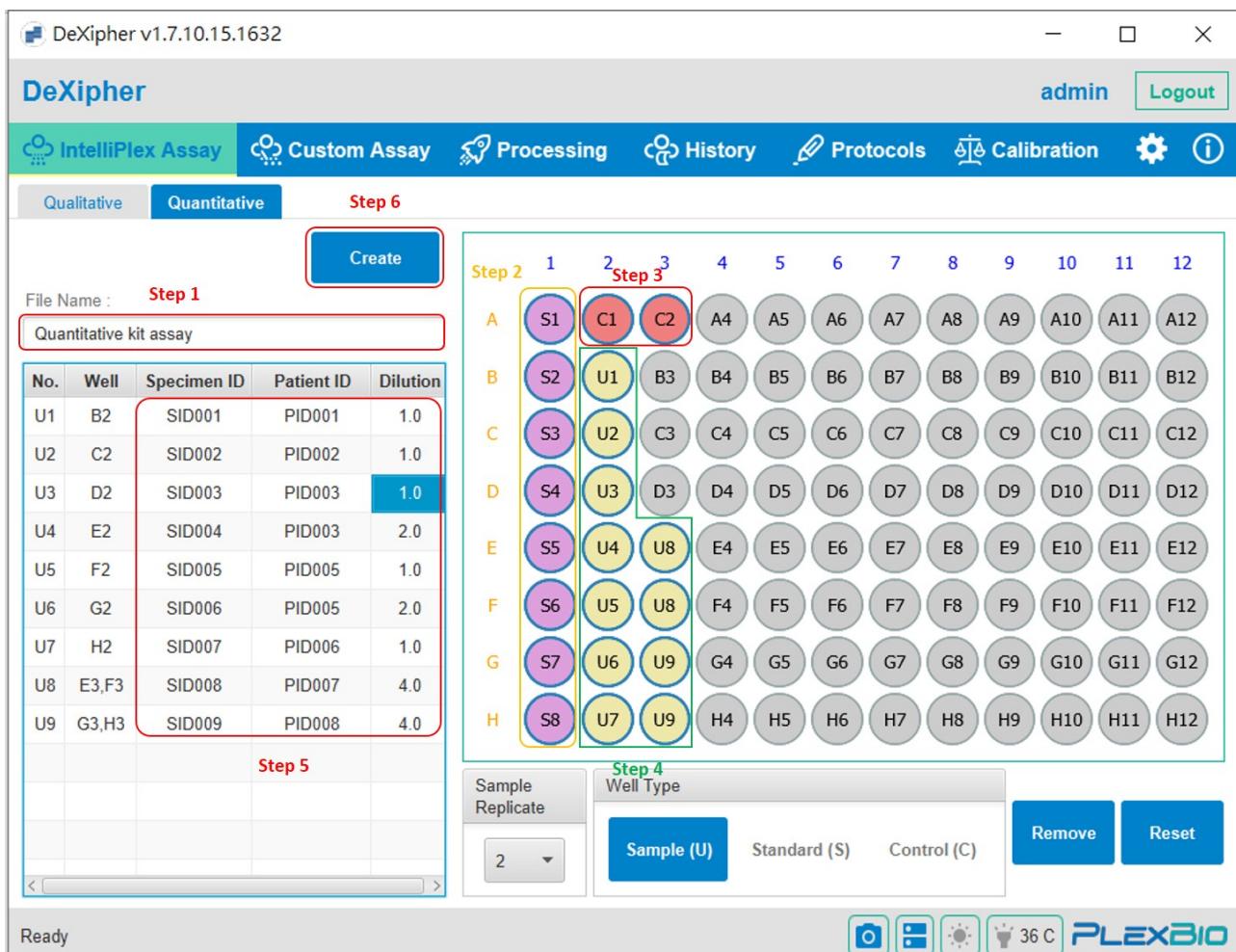


Fig. 17. IntelliPlex Assay - Quantitative

- Step 1. Enter **File Name** (Example: “Quantitative kit assay”).
- Step 2. Select **Standard** wells (S1 to S8, well A1 to H1; purple).
- Step 3. Select **Control** wells (C1 and C2, well A2 and A3; red).
- Step 4. Select **Sample** wells (U1-U7 with Sample Replicate = 1, wells B2-H2; sample U8 and U9 with Sample Replicate = 2, wells E3 and F3 for sample U8 and wells G3 and H3 for sample U9; yellow wells).
- Step 5. Enter Dilution Factor of each sample (“Dilution”). Optional: Enter Specimen ID and Patient ID. The plate format is available as reference.
- Step 6. Click “Create” to proceed to the **Processing tab** (go to step 7 below).

Note: All information has been saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can be edited or deleted before reading.

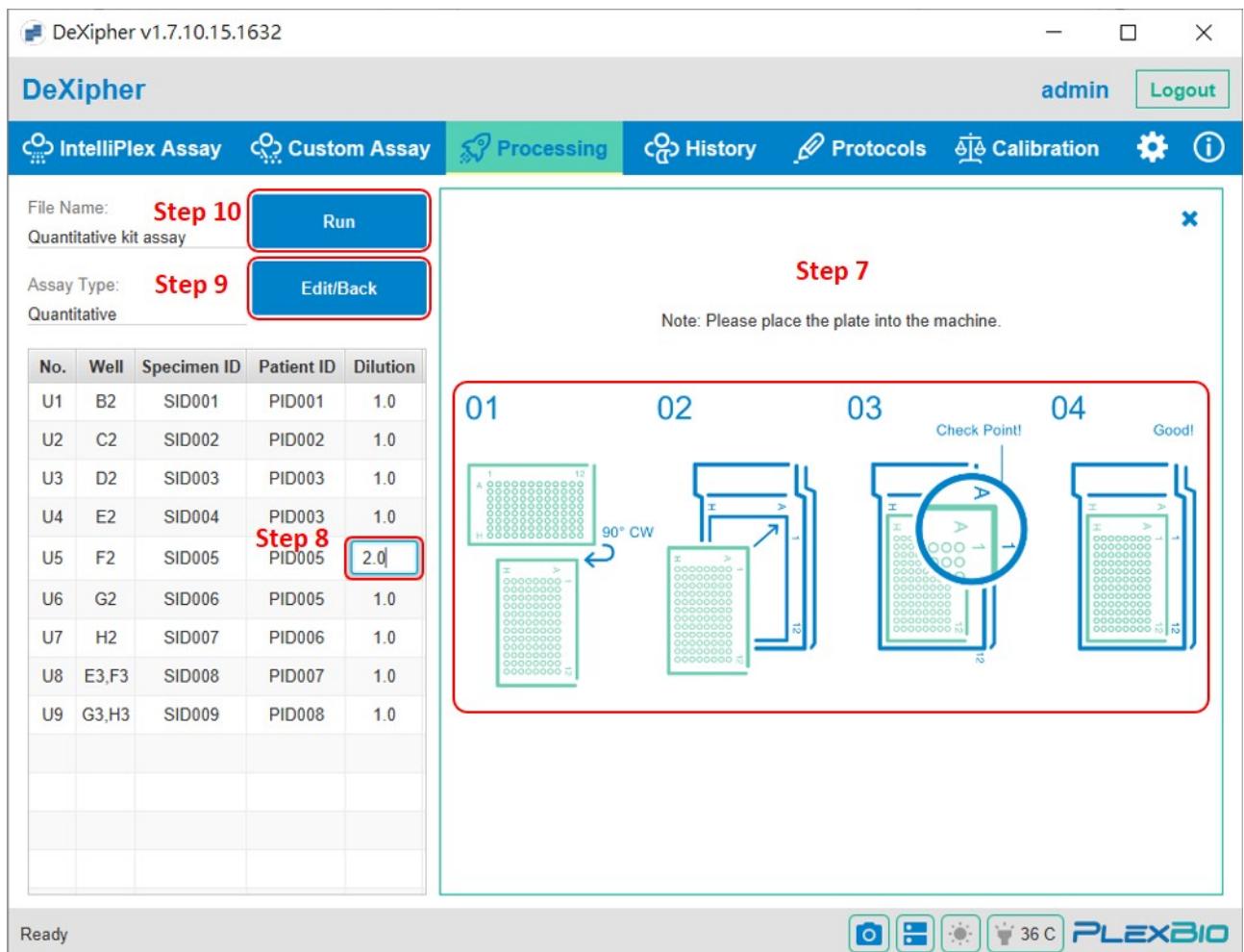


Fig. 18. IntelliPlex Assay - Processing

- Step 7. Follow the drawing to put the 96-well plate onto the stage (plate holder) with the correct orientation (Fig. 18).
- Step 8. Specimen information can be entered prior, during or after the reading. Ensure **Dilution Factor** of each sample is correct. The default value is “1”.
To see the plate with sample selection, remove the plate instruction by clicking the “X” on the top right corner.
- Step 9. Click the “Edit/Back” button to change sample selection in the 96-well plate.
- Step 10. Click “Run” to start reading.

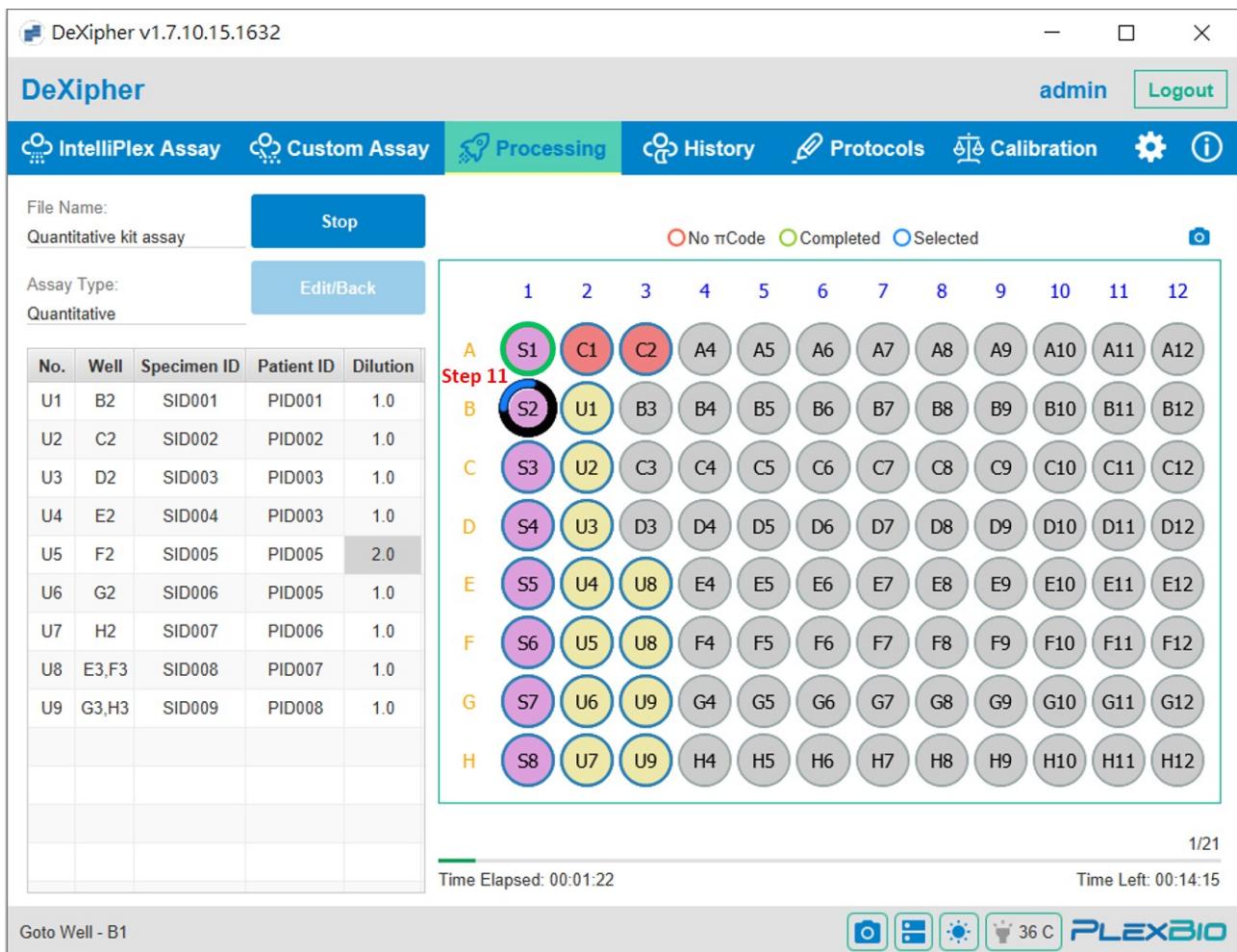


Fig. 19. IntelliPlex Assay - Processing - Run Status

- Step 11. PlexBio 100 Fluorescent Analyzer starts to read selected wells (Fig. 19).
- Step 12. Wells that are being read are circled black with a blue area moving around the circle (It takes approximately 50 sec to read one well). The camera symbol on the top right corner of the 96-well plate allows to see a live image of the well being analyzed.
- Step 13. Wells that have not been analyzed are circled blue (=selected).
- Step 14. Wells that have been analyzed are circled green (=complete) if πCode were recognized.
- Step 15. Wells that have been analyzed are circled red (=No πCode) if DeXipher was unable to recognize πCode in the well.

Note: Do not open the Lid while the PlexBio 100 Fluorescent Analyzer is analyzing wells. Opening the lid will cause a hard abort and terminate the analysis. The analysis cannot be resumed, and the results of unread wells will not be available. If – for any reason – a read needs to be stopped before it is completed, click **Stop** instead of opening the lid to terminate the analysis. The analysis still cannot be resumed, and the results of unread wells will not be available, but PlexBio 100 Fluorescent Analyzer will move the stage to its default position, where the 96-well plate can be taken out. Wait until DeXipher confirms that the run has been successfully terminated before opening the lid.

- Step 16. Once DeXipher has analyzed all wells, a pop-up window will confirm that the read is completed.
- Step 17. Sample information can be added until the file has been confirmed and the status is *FINISHED*. Note: Do not confirm a file if the assay-lot-specific ENC file is not imported (Refer to chapter 8 for more information on how to confirm and change status to *FINISHED*).
- Step 18. All results are saved to the file and can be accessed at any time in the **History tab** (chapter 9). For each confirmed file, a report of the result will also be available.

Chapter 7. Custom Assay

The **Custom Assay tab** is available in DeXipher RUO and Basic. For DeXipher Basic, the **Custom Assay tab** only includes *Quick Read*. For DeXipher RUO, the **Custom Assay tab** includes the functions *Quick Read*, *Qualitative* and *Quantitative*.

The *Quick Read* option analyzes the πCode image patterns and corresponding fluorescence signals, but does not apply any protocols. *Quick Read* does not support different well types, all wells are analyzed independent from each other. Results for each well include the fluorescence signal for each analyzed πCode and the median fluorescence signal for each group of πCode with the same image pattern. Refer to section 7.1 for more details.

The *Qualitative* option (DeXipher RUO only) analyzes a qualitative assay based on selected protocol and supports the well types Positive Control (PC), Negative Control (NC), and Unknown (U). The results will be calculated based on the selected protocol. Refer to section 7.2 for more details.

The *Quantitative* option (DeXipher RUO only) analyzes a quantitative assay based on selected protocol and supports the well types Standard (S), Control (C), and Unknown (U). The results will be calculated based on the selected protocol. Refer to section 7.2 for more details.

The PlexBio 100 Fluorescent Analyzer needs monthly calibration. Refer to chapter 11 for more details on how to calibrate PlexBio 100 Fluorescent Analyzer.

Refer to chapter 10 regarding setup, import and export of protocols.

Note: Any protocol can retroactively be applied to results obtained using any of the three options in the **Custom Assay tab**, including *Quick Read*. The results must fulfill the protocol requirements (such as using the πCode image pattern defined in the protocol, include wells for PC/NC or Standards, etc.) to allow applying a protocol successfully. Refer to chapter 9 for more information.

7.1 Quick Read

Quick Read allows reading wells without any additional setup such as defining the πCode MicroDiscs identities in the well or defining the well type as control/standard/sample. Quick Read only has one well type (R). Applications for Quick Read include:

- To determine the identity and fluorescence intensity of πCode MicroDisc in a well.
- To quickly check the distribution of πCode MicroDisc in a well.
- To count the πCode Microdisc in a well.

Quick Read is designed to allow fast and convenient access to the πCode MicroDiscs information in a well. The function supports the development of new assays. *Quick Read* analyzes any well without the need to add additional information. Results can be exported as an XLSX file to facilitate easy and convenient analysis. The exported XLSX file contains all identified πCode MicroDiscs and their respective measured fluorescent intensity as well as the median fluorescent intensity of πCode MicroDiscs grouped by identity.

To setup up and run a Quick Read, the following information is required:

- **File Name:**

Provide a unique name for the analysis. If there is no name or the name has already been used the “Create” button will remain disabled.

- **Well Selection:**

Quick Read does not support “well type” selection. All wells are considered “samples”. Mark all wells that require analysis in the schematic display of a 96-well plate (marked wells are highlighted in white). To select multiple wells with samples, keep the left mouse button pressed while moving over all wells that contain Unknowns.

Note: Load 96-well plates lane-wise. The IntelliPlex-technology processes 96-well plates by lanes, which are defined by the 8 well strip going from A to H. A 96-well plate consists of 12 lanes (1-12); each lane consists of 8 wells (A-H).

Remove:

To remove a well selection, mark each well that needs to be cleared (marked wells will be highlighted with a red box) and click Remove.

Reset:

To reset the whole plate (remove all entries), click Reset.

Sample Information:

Provide information such as Specimen ID and Patient ID for each well containing a sample. Providing this information in the **Quick Read sub-tab** is optional. The information can also be added in the **Processing tab** before, during or after the read. Sample information can be added/ edited until the file is confirmed, and the status is set to *FINISHED* (chapter 8).

Create:

After entering a valid **File Name** and selecting all wells, the “Create” button will be active. Click on it to save the entered information and to proceed to **Processing tab** to read the plate.

All information has been saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can be edited or deleted before reading.

Editing a file:

Any created file that has not been analyzed can be edited by clicking “Edit/Back” in the **Processing tab** (chapter 8). DeXipher will return to the **Quick Read tab** and allow updating the well selection and File Name. To confirm or discard changes, the following options are available:

- **Update:**

To save modification and update the original created file and proceed to the **Processing tab**, click “Update”.

- **New:**

To abort editing and discard all changes, click “New”. The content of the original created file will not be modified and can be accessed in the **History tab**.

- **Delete:**

To delete a created file permanently, click “Delete”. The file and all information associated with it will be deleted and can no longer be received in the **History tab**.

Example to setup and run a Quick Read (Fig.20):

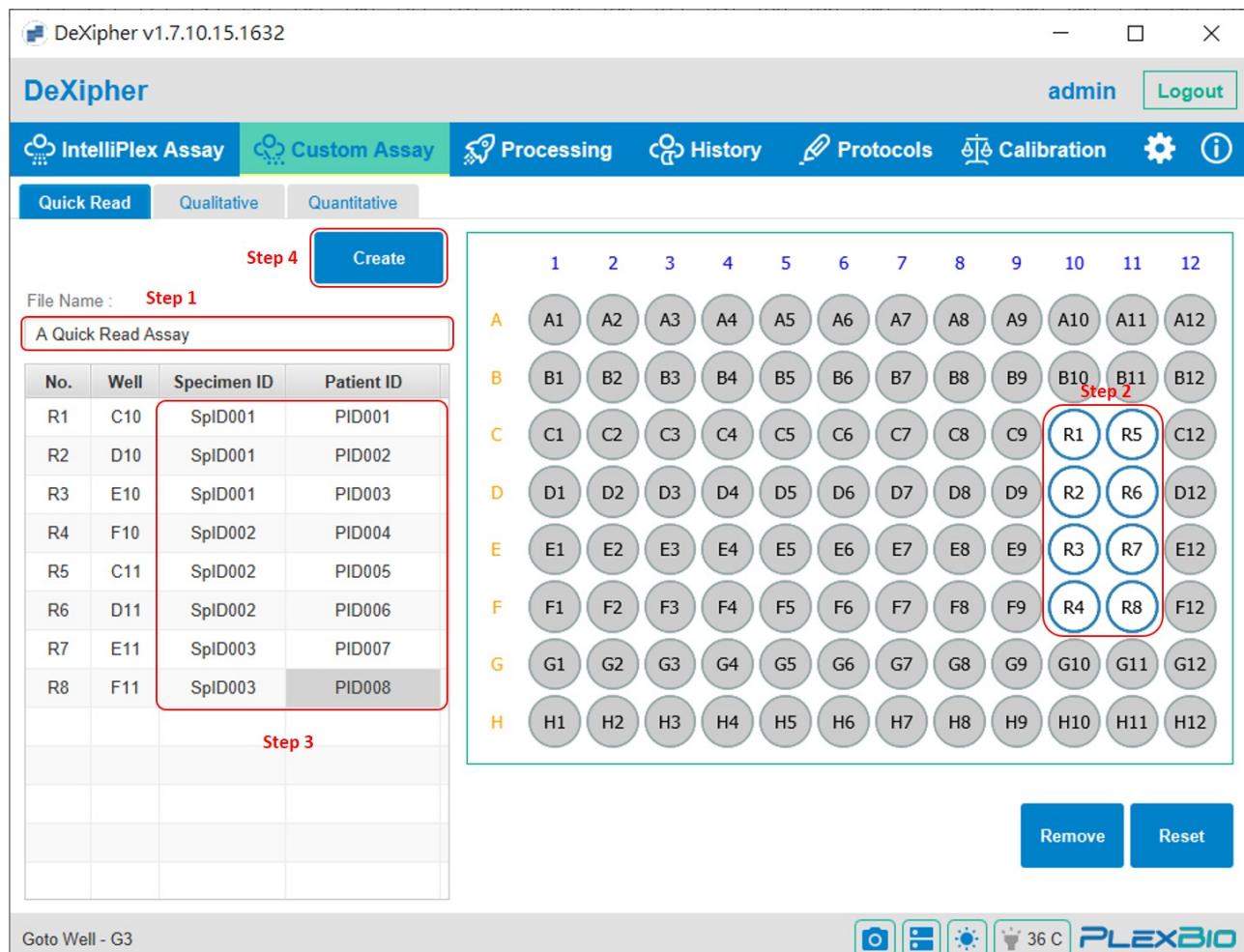


Fig. 20. Quick Read Screen

- Step 1. All Quick Reads require a unique **File Name**.
- Step 2. Select wells by clicking or dragging the cursor (marked wells are highlighted in white). Well selection can be removed using the “Remove” function. Users can click “Reset” to remove all selected wells and start over.
- Step 3. Optional: Enter sample descriptions. The plate format is available as reference.
- Step 4. Click “Create” to proceed.

Note: All information has been saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can be edited or deleted before reading.

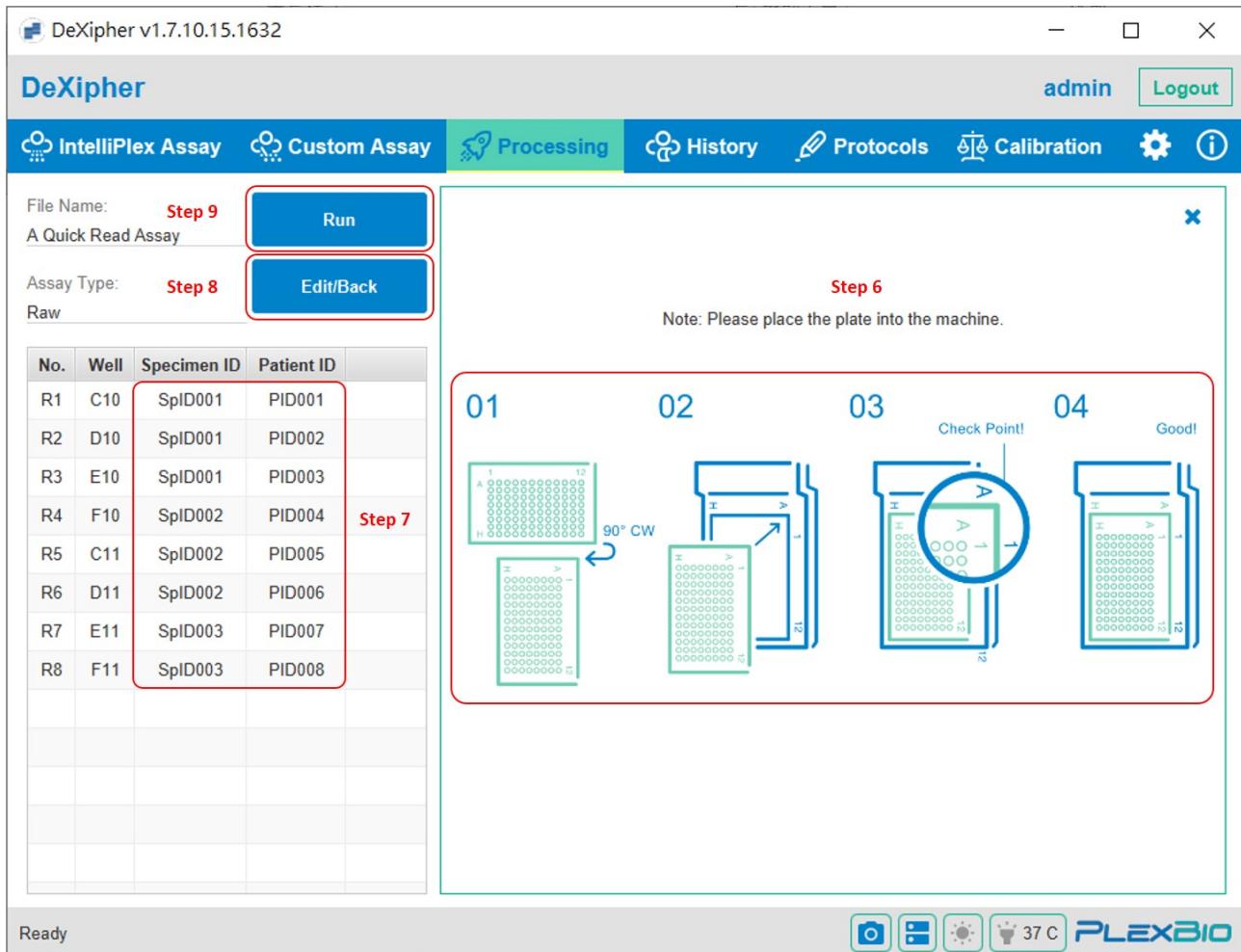


Fig. 21. Quick Read/Processing

- Step 5. The **Processing tab** will be activated (Fig. 21).
- Step 6. Follow the drawing to put the 96-well plate onto the stage (plate holder) with the correct orientation.
- Step 7. Specimen information can be entered prior, during or after the reading. To see the plate with sample selection, remove the plate instruction by clicking the “X” on the top right corner.
- Step 8. Click the “Edit/Back” button to change sample selection in the 96-well plate.
- Step 9. Click “Run” to start reading.

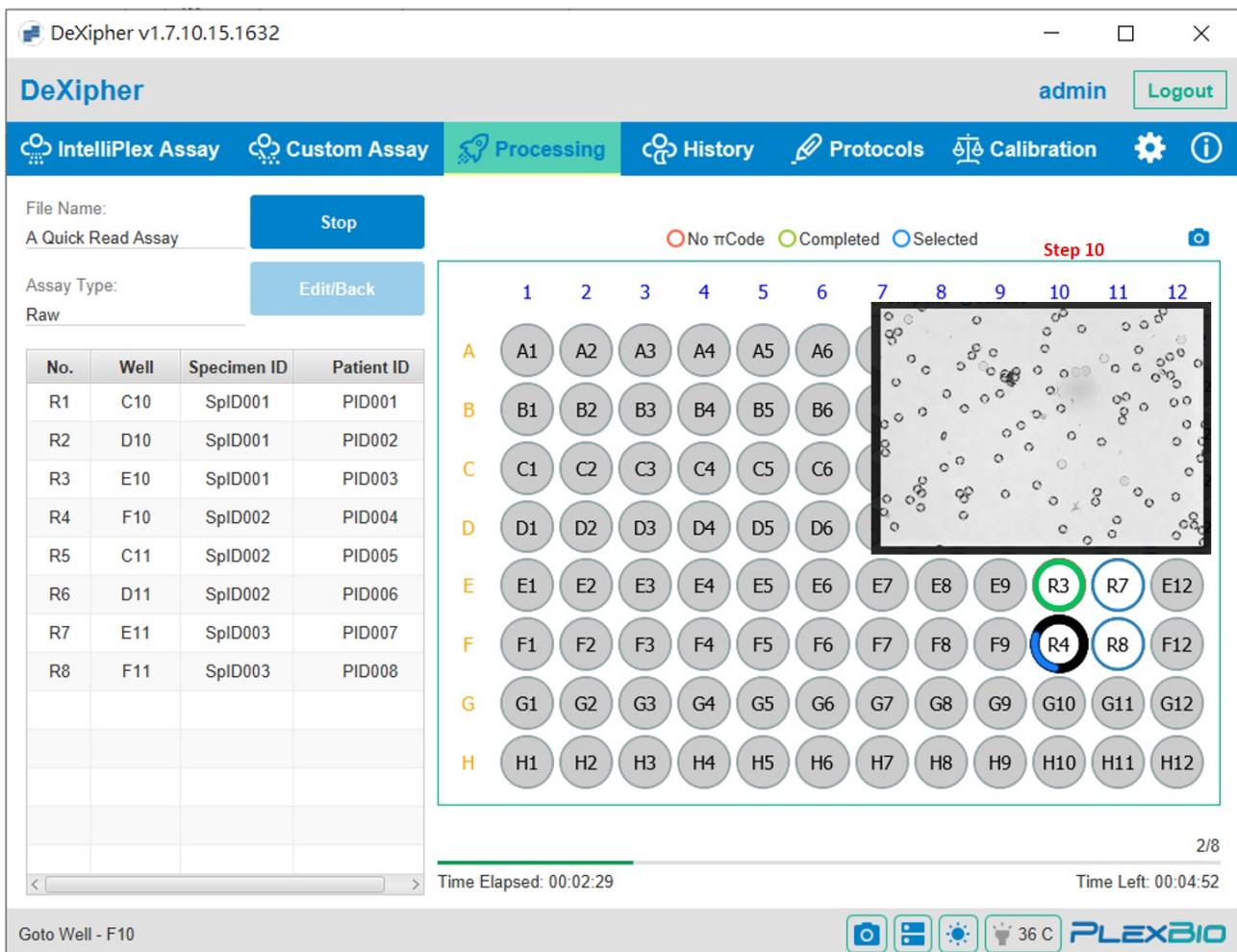


Fig. 22. Run screen during reading

- Step 9. PlexBio 100 Fluorescent Analyzer starts to read selected wells (Fig. 22).
- Step 10. Wells that are being read are circled black with a blue area moving around the circle (It takes approximately 50sec to read one well). The camera symbol on the top right corner of the 96-well plate allows to see a live image of the well being analyzed.
- Step 11. Wells that have not been analyzed are circled blue (=selected).
- Step 12. Wells that have been analyzed are circled green (=complete) if πCode were recognized.
- Step 13. Wells that have been analyzed are circled red (=No πCode) if DeXipher was unable to recognize πCode in the well.

Note: Do not open the Lid while the PlexBio 100 Fluorescent Analyzer is analyzing wells. Opening the lid will cause a hard abort and terminate the analysis. The analysis cannot be resumed, and the results of unread wells will not be available. If – for any reason – a read needs to be stopped before it is completed, click **Stop** instead of opening the lid to terminate the analysis. The analysis still cannot be resumed, and the results of unread wells will not be available, but PlexBio 100 Fluorescent Analyzer will move the stage to its default position, where the 96-well plate can be taken out. Wait until DeXipher confirms that the run has been successfully terminated before opening the lid.

- Step 14. Once DeXipher has analyzed all wells, a pop-up window will confirm that the read is completed.
- Step 15. Sample information can be added until the file has been confirmed and the status is FINISHED (refer to chapter 8 for more information on how to confirm and change status to *FINISHED*).
- Step 16. All results are saved to the file and can be accessed at any time in the **History tab** (chapter 9).

7.2 Quantitative or Qualitative

The **Custom Assay tab - Quantitative or Qualitative** (DeXipher RUO only) allows the User to develop and use their own protocols and assays. Refer to chapter 10 for more information on custom protocols. Depending on whether *Quantitative or Qualitative* is selected, different information needs to be provided to create a file and start a run.

DeXipher will automatically identify and analyze the πCode image patterns and corresponding fluorescence signals in each well and correlate the information with the protocol requirements. Results can be accessed as raw data and exported as XLSX file format. The exported XLSX file contains all identified πCode MicroDiscs and their respective measured fluorescent intensity as well as the median fluorescent intensity of πCode MicroDiscs grouped by identity. The Custom Assay - *Quantitative or Qualitative* files support generation of reports (based on the selected protocol).

To setup up and run a *Quantitative or Qualitative* Read, the following information in the following order is required. Note that wells can only be selected after the **File Name** and the **Protocol** are selected.

File Name: Provide a unique name for the analysis.

Protocol: Select desired protocol from the drop-down menu.

Add Protocol: Click to create and add a new protocol. Refer to chapter 10 for more details.

Well Selection: The “**Well Type**” will depend whether *Quantitative or Qualitative* is selected. Mark all wells as required in the schematic display of a 96-well plate.

Quantitative:

Select the type of sample and the position in the 96-well plate by selecting the “**Well Type**” (U = Sample (Unknown), S = Standard, C = Control) first, and then the position in the schematic display of a 96-well plate.

Protocols require the inclusion of a series of standards (the number of standards required for selected protocol is shown in the red circle in Standard box; selected wells marked in purple) and control(s) (the number of controls required for selected protocol is shown in the red circle in Control box; selected wells are marked in grey). Selected sample wells are marked in yellow.

To select the sample, standard or control in duplicate (or higher replication), select the appropriate number from the Sample Replicate box. The replicate number for each sample can differ (e.g. duplicate reading for sample 1, single read for sample 2 etc.), but the replicate number for all standards and controls must be the same (e.g. all standards read as duplicates, all controls read as triplicates etc.).

To select multiple wells at once, select multiple wells while keeping the left mouse button clicked. Samples will be filled lane-wise (A-H). For example, if 2 replicates for samples are selected, selecting all wells in lane 1 and 2 will associate sample 1 to well A1 and B1, sample 2 to wells C1 and D1 all the way to sample 8 in wells G2 and H2.

Qualitative:

Select the type of sample and the position in the 96-well plate by selecting the “**Well Type**” (U = Sample (Unknown), PC = Positive Control, NC = Negative Control) first, and then the position in the schematic display of a 96-well plate.

The exact requirements for PC (Positive Control =selected wells are marked in red) and NC (Negative Control, selected wells are marked in grey) for each assay depend on the selected protocol. Selected wells for Unknown are marked in yellow. To select multiple wells with samples, keep the left mouse button pressed while moving over all wells that contain Unknowns.

Note: Load 96-well plates lane-wise. The IntelliPlex-technology processes 96-well plates by lanes, which are defined by the 8 well strip going from A to H. A 96-well plate consists of 12 lanes (1-12); each lane consists of 8 wells (A-H).

Sample Information:

Provide information such as Specimen ID and Patient ID for each sample containing well. For Quantitative assays, add Dilution factor. **The Dilution Factor is set to “1” by default and needs to be adjusted accordingly for each sample.** Providing this information in the **Custom Assay tab** is optional. The information can also be added in the **Processing tab** before, during or after the read. Sample information can be added/ edited until the file is confirmed, and the status is set to *FINISHED* (chapter 8).

Create:

After entering a valid **File Name** and selecting wells, the “Create” button will be activated. Click on it to save the entered information and to proceed to **Processing tab** to read the plate.

All information has been saved to the File Name and can be retrieved in the **History tab** to read the assay at any time. The file can be edited or deleted before reading.

Note: The **Custom Assay Quantitative or Qualitative** function allows to select only one protocol

during setup. Users who want to read 96-well plates with samples belonging to different protocols can read all wells with the *Quick Read* function (section 7.1) and apply different protocols to a subset of samples using the “Apply Protocol” function in the **History tab** (chapter 9).

Editing a file:

Any created file that has not been analyzed can be edited by clicking “Edit/Back” in the **Processing tab** (chapter 8). DeXipher will return to the *Quantitative or Qualitative* sub-tab of the **Custom Assay tab** and allow updating the well selection and File Name. To confirm or discard changes, the following options are available:

- **Update:**

To save modification and update the original created file and proceed to the **Processing tab**, click “Update”.

- **New:**

To abort editing and discard all changes, click “New”. The content of the original created file will not be modified and can be accessed in the **History tab**.

- **Delete:**

To delete a created file permanently, click “Delete”. The file and all information associated with it will be deleted and can no longer be received in the **History tab**.

Example to setup and run *Custom Assay - Qualitative* (Fig. 23):

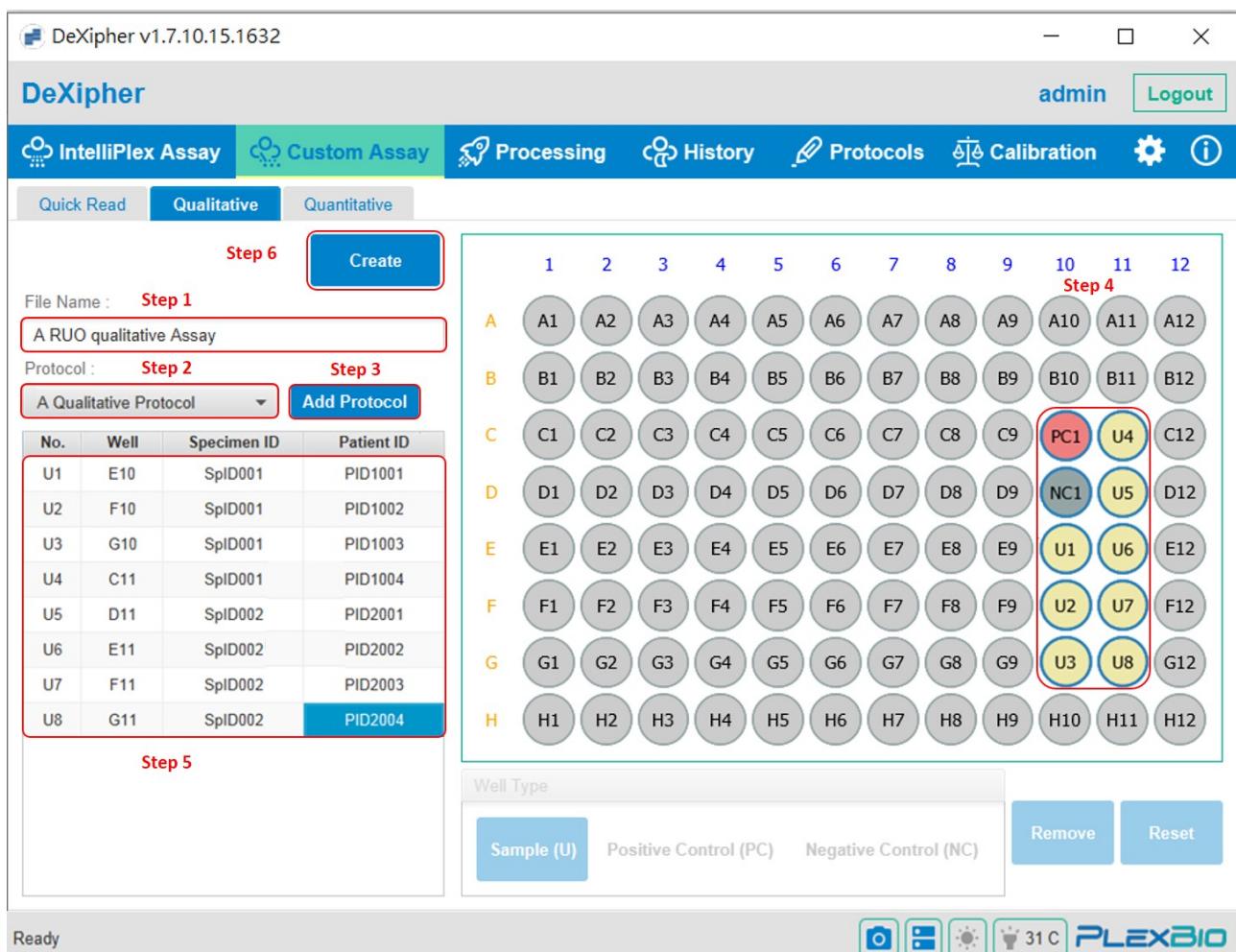


Fig. 23. Custom Assay – Qualitative

- Step 1. All runs require a unique **File Name**.
- Step 2. All runs require selection of a **Protocol**.
- Step 3. To create a new protocol, select “Add Protocol” or change to the **Protocol tab** (chapter 10).
- Step 4. Select wells with appropriate “Well type” as required by the protocol (for Custom Assay – Qualitative: Positive Control (PC), Negative Control (NC) and Sample (U); for Custom Assay – Quantitative: Standard (S), Control (C) and Sample (U)). Select the number of replicates as appropriate. Wells can be selected one-by-one or as multiple well selection.
- Step 5. For **Custom Assay – Quantitative**: Enter Dilution Factor of each sample (“Dilution”). Optional: Provide additional sample information (Custom Assay – Qualitative/Quantitative).
- Step 6. Click “Create” to proceed.

Note: All information has been saved to the File Name and can be retrieved in the **History** tab to read the assay at any time. The file can be edited or deleted before reading.

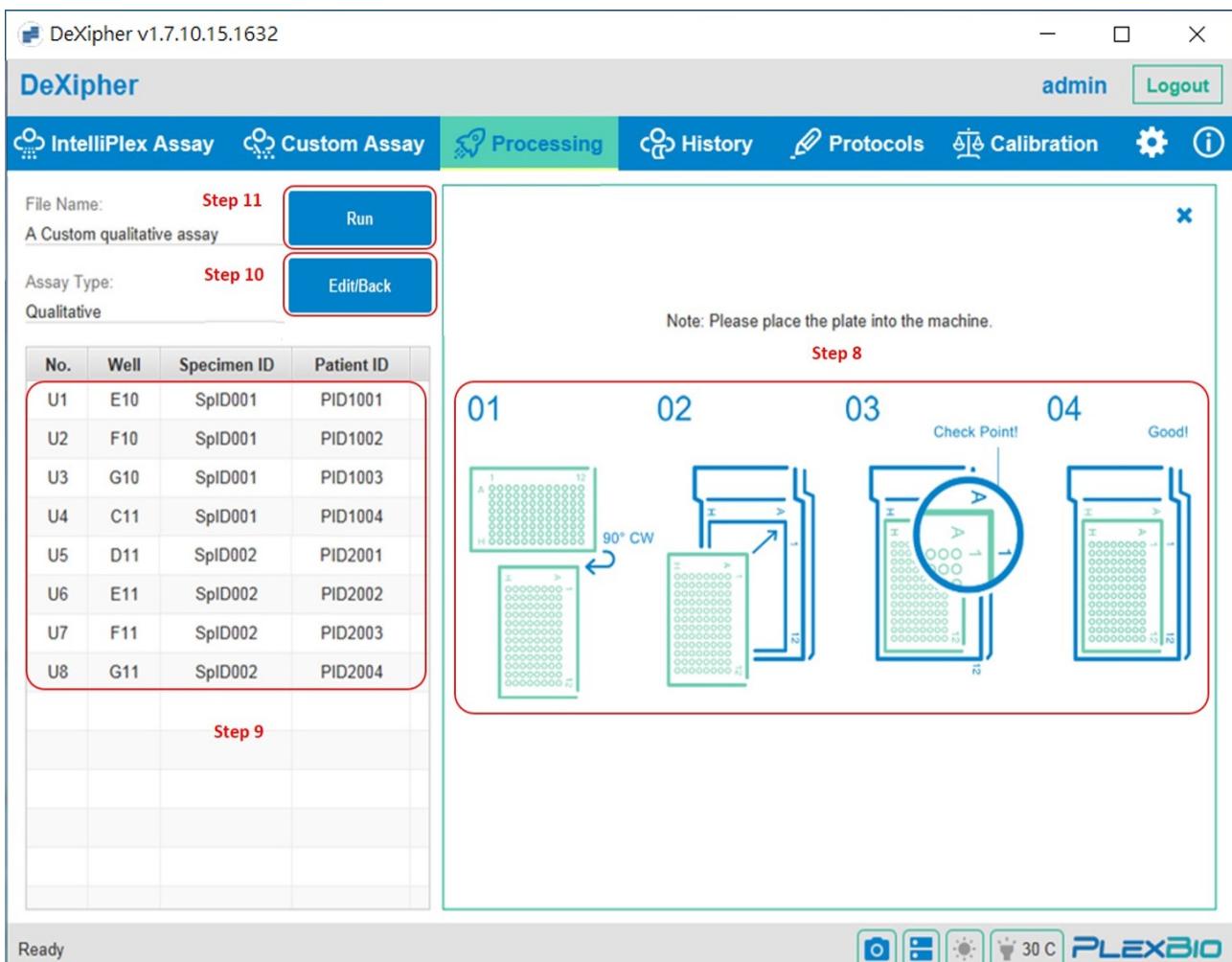


Fig. 24. Custom Assay - Qualitative - Processing

- Step 7. The **Processing** tab will be activated (Fig. 24).
- Step 8. Follow the drawing to put the 96-well plate onto the stage (plate holder) with the correct orientation.
- Step 9. Specimen information can be entered prior, during or after the reading.
For **Custom Assay – Quantitative**: Ensure **Dilution Factor** of each sample is correct. The default value is “1”.
To see the plate with sample selection, remove the plate instruction by clicking the “X” on the top right corner.
- Step 10. Click the “Edit/Back” button to change sample selection in the 96-well plate.
- Step 11. Click “Run” to start reading.

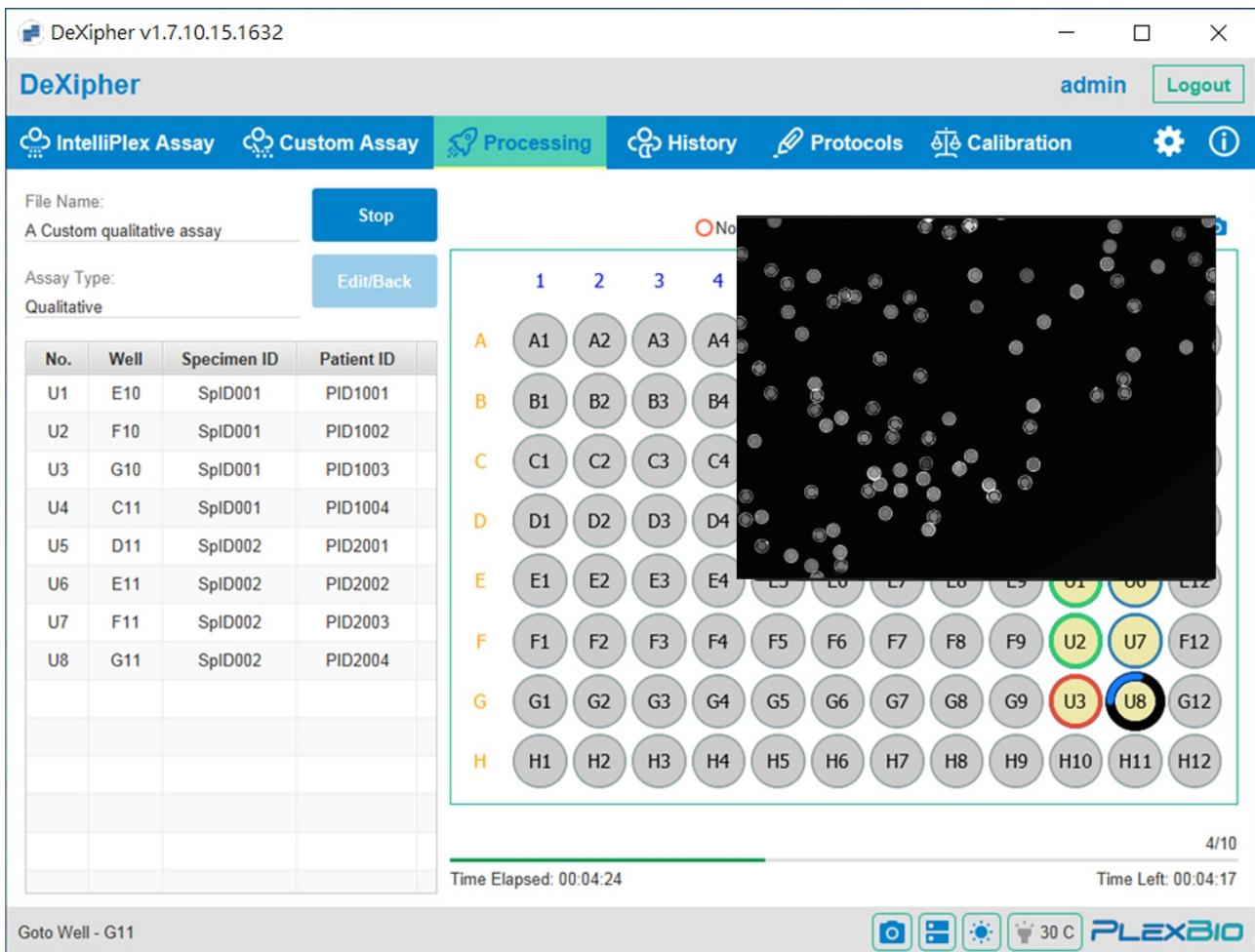


Fig. 25. Processing screen during reading

- Step 12. PlexBio 100 Fluorescent Analyzer starts to read selected wells (Fig. 25).
- Step 13. Wells that are being read are circled black with a blue area moving around the circle (It takes approximately 50sec to read one well). The camera symbol on the top right corner of the 96-well plate allows to see a live image of the well being analyzed.
- Step 14. Wells that have not been analyzed are circled blue (=selected).
- Step 15. Wells that have been analyzed are circled green (=complete) if πCode were recognized.
- Step 16. Wells that have been analyzed are circled red (=No πCode) if DeXipher was unable to recognize πCode in the well.

Note: Do not open the Lid while the PlexBio 100 Fluorescent Analyzer is analyzing wells. Opening the lid will cause a hard abort and terminate the analysis. The analysis cannot be resumed, and the results of unread wells will not be available. If – for any reason – a read needs to be stopped before it is completed, click **Stop** instead of opening the lid to terminate the analysis. The analysis still cannot be resumed, and the results of unread wells will not be available, but PlexBio 100 Fluorescent Analyzer will move the stage to its default position, where the 96-well plate can be taken out. Wait until DeXipher confirms that the run has been successfully terminated before opening the lid.

- Step 17. Once DeXipher has analyzed all wells, a pop-up window will confirm that the read is completed.
- Step 18. Sample information can be added until the file has been confirmed and the status is *FINISHED* (Refer to chapter 8 for more information on how to confirm and change status to *FINISHED*).
- Step 19. All results are saved to the file and can be accessed at any time in the **History tab** (chapter 9). For each confirmed file, a report of the result will also be available.

Chapter 8. Processing Tab

The **Processing tab** is available in all DeXipher license types.

The **Processing tab** serves to start and follow the analyzing process of selected samples in the 96-well plate. Before analyzing a plate, the **Processing tab** offers the option to edit selected wells and well types. Depending on the status of the assay, accessible functions in the **Processing tab** may vary. An overview is shown in the scheme below:

Assay status				
FILE STATUS	EDIT_COMPLETED	ASSAYING	ASSAYED	FINISHED
Discard an existing file	✓			
Add/Change the well to be read.	✓			
Add/Change the Well Type	✓			
Edit sample information	✓	✓	✓	
View well image		✓	✓	✓
View/Export the raw data of a single well		✓	✓	✓
Use other tabs	✓	✓	✓	✓

Before analyzing a plate, the **Processing tab** allows to edit the file information and to start a run/read (section 8.1). The **Processing tab** allows to look at and to export results of any single well that has been analyzed (section 8.2).

Files can be “confirmed” in the **Processing tab** (section 8.3), which will change the file status to **FINISHED**. Confirming an IntelliPlex-branded assay file or a Quantitative or Qualitative file will grant access to the report. Note that confirmation of IntelliPlex-branded assays requires assay-specific APP and lot-specific ENC file to be installed.

Note: Access and resume previously created files from the **History tab** (chapter 9) for further processing in the **Processing tab**.

8.1 Edit and Run a File

The **Processing tab** is used to analyze wells in a 96-well plate with the PlexBio 100 Fluorescent Analyzer.

A file is created in the **IntelliPlex Assay tab** (chapter 6) or the **Custom Assay tab** (chapter 7) and then analyzed in the **Processing tab**. Any created files can be accessed in the **History tab** (chapter 9) for further processing in the **Processing tab**.

To insert a plate, follow the drawing shown in the **Processing tab**.

To see the well and well type selection, click on the “X” on the top right edge of the window instructing plate insertion into the PlexBio 100 Fluorescent Analyzer. Clicking the “X” removes the drawing and displays the 96-well plate with well selection.

To analyze a plate according to the specified well and sample type selection, press “Run” (If well selection or sample type needs editing before analyzing, see “Edit/Back” function below). Ensure that the 96-well plate is correctly inserted in the PlexBio 100 Fluorescent Analyzer:

- Selected wells that have not been analyzed are circled blue.
- Wells that are actively being analyzed are circled black with a blue dot moving around the circle.
- Wells that have been analyzed are circled green if πCode MicroDiscs were recognized.
- Wells that have been analyzed are circled red if DeXipher was unable to recognize πCode MicroDiscs in the well.

Note for wells actively being analyzed: The camera symbol on the top right corner of the 96-well plate allows to see a live image of the pictures as they are taken.

To edit sample information such as Specimen ID and Patient ID, edit the table in the **Processing tab**. Sample information can be edited before, during and after the wells are analyzed by the PlexBio 100 Fluorescent Analyzer. Sample information can no longer be edited once the results have been confirmed. See section 8.3 for more details.

Before analyzing an assay, all well related information can be edited using the “Edit/Back” function. The screen will return to the setup screen which allows to update the file name, sample and well selection. To complete or abort editing, the following options are available:

- **Update:** To save modification and update the original created file and proceed to the **Processing tab**, click “Update”.

- **New:** To abort editing and discard all changes, click “New”. The content of the original created file will not be modified and can be accessed in the **History tab**.
- **Delete:** To delete a created file permanently, click “Delete”. The file and all information associated with it will be deleted and can no longer be received in the **History tab**.

8.2 Analyze/Export Data from a Single Well

The results of a green circled well can be accessed by double clicking the well.

A pop-up window opens, showing the bright field image of the well. The well is composed of 21 frames. The window will also show a table of results, listing the identity and quantity of identified πCode MicroDiscs and median fluorescence intensity.

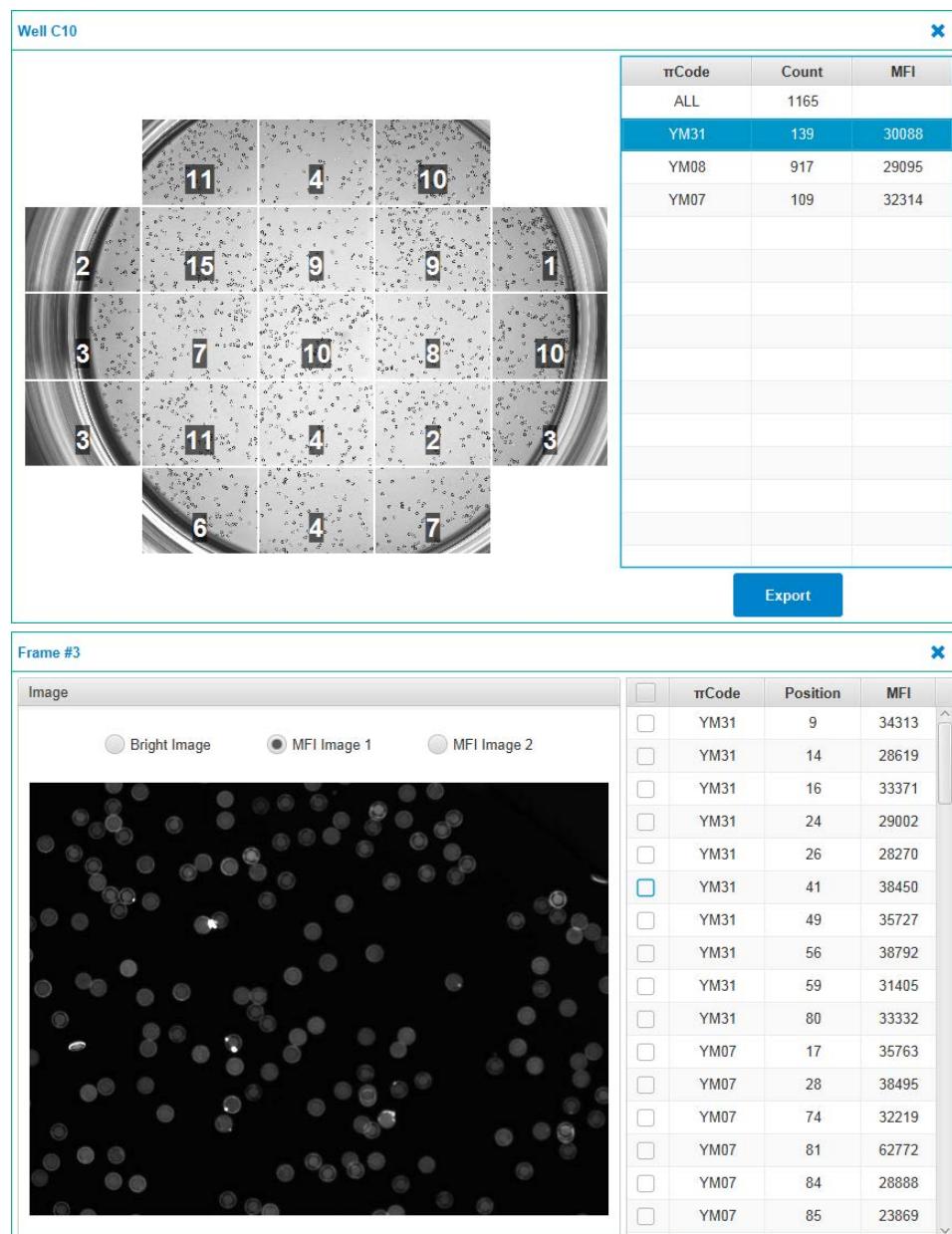


Fig. 26. Pop-up windows to show processing result

Clicking on any of the πCode Identities in the table will show the distribution of that πCode Identity in the well. The number displayed in each of the 21 frames reflects the count of the selected πCode MicroDisc identity in that frame.

By clicking inside any of the frames, the frame will be enlarged and the “Bright Image” (Bright Field Image) or “MFI Image 1” or “MFI Image 2” (Fluorescence Image, long and short exposure) can be selected. Each identified πCode on the screen can be selected (by clicking on it) and the selected πCode will be linked to the information in the overview table to the right. Likewise, selecting a πCode in the table will show the position in the well. Multiple πCode can be selected at the same time. The πCode selection can be deselected by unchecking the corresponding box in the table.

The opened frame and the well pop-up window can be closed by clicking the “X” on the top right corner of the pop-up window.

The **Export** button allows to export the results for the selected well as XLSX file.

8.3 Confirming Results – Set Status to FINISHED

Refer to section 9.1 for more details on the file status system used by DeXipher. Note that once a file is confirmed, sample information such as Specimen ID, Patient ID or Dilution Factor can no longer be edited. Only confirm files once all information has been correctly added.

Results can be accessed and exported as raw data and summary data (XLSX format) for any file that has been analyzed. To access reports, the file must be confirmed, which will finalize the file and set its status to *FINISHED*. A file can be “confirmed” at any time after the PlexBio 100 Fluorescent Analyzer completed the analysis (Fig. 27).

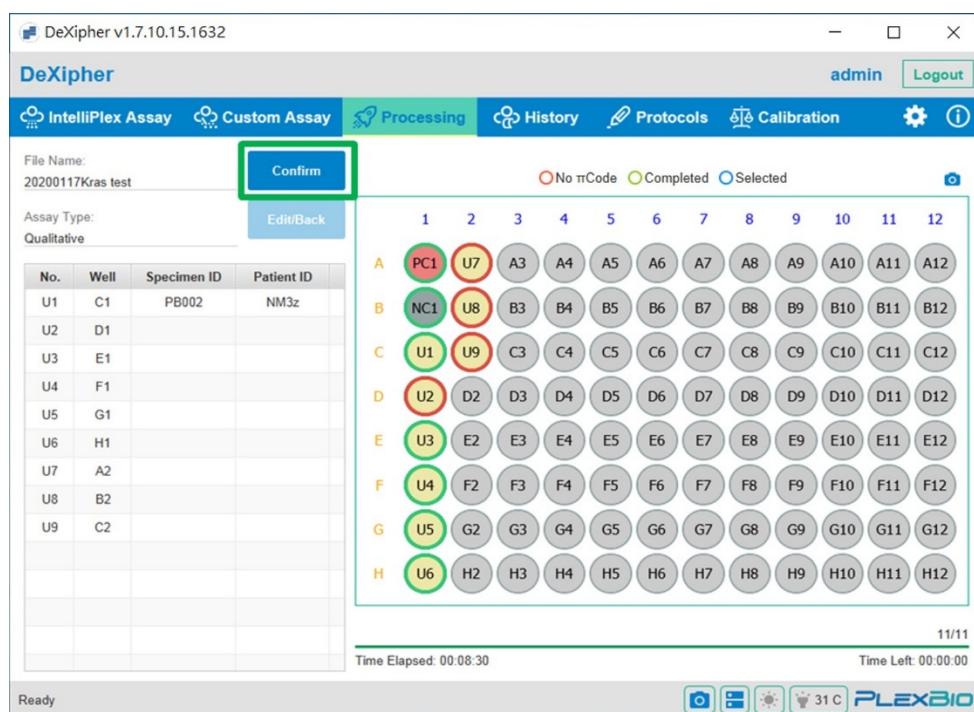


Fig. 27. Confirming of a completed analysis

- **Custom Assay – Quick Run**

Confirming a Custom Assay – *Quick Run* file will lock all sample related information, which can no longer be edited. Once the file is confirmed, the status will be *FINISHED*. There are no PDF reports associated with Quick Read files per se, but protocols can be applied to *FINISHED* files (chapter 9).

- **Custom Assay –Qualitative or Quantitative**

Confirming a Qualitative or Quantitative assays will lock all sample related information, which can no longer be edited. Once *FINISHED*, a report will be generated. Additional protocols can be applied (chapter 9). **The dilution factor for all samples in Custom Assay - Quantitative is set to “1” by default** and must be adjusted before confirming the file. Once confirmed, the dilution factor cannot be changed anymore. Concentration will be calculated based on the entered dilution factor.

- **IntelliPlex Assay**

Confirming a file related to an IntelliPlex-branded assay **requires the assay-lot-specific ENC file to be imported**. Without the ENC file, assay-lots will not be recognized by the software. There will be a warning during the confirmation process if the assay lot is not recognized for one or more samples. The ENC file can be downloaded from PlexBio official website.

All sample must have a Specimen ID to confirm a file. If Specimen ID is missing, a warning window will pop-up and instruct to fill out the missing Specimen IDs. **The dilution factor for all samples in Quantitative Assays is set to “1” by default** and must be adjusted before confirming the file. Once confirmed, the dilution factor cannot be edited anymore. Concentration will be calculated based on the provided dilution factor.

Once the file is confirmed, the status will be *FINISHED* and sample information can no longer be edited. Once *FINISHED*, a report will be generated. Additional protocols can be applied (chapter 9).

Follow the step-by-step instruction to confirm IntelliPlex-branded assay:

Step 1. Ensure that for all samples, the Specimen ID information is added.

Step 2. Click the “Confirm” button.

Step 3. A pop-up window, “Kit Mapping Summary”, with the summary of kit mapping results, including kit name(s) and lot number(s) of the assay(s) will appear (Fig. 28). The table shows all recognized kits (assay-lot specific ENC imported) with their corresponding wells. The table also shows “Unknown Wells” for all wells that could not be matched to an ENC file (see “Note” below) or “Error Wells” (π Code MicroDisc not found). If all wells associated to a specific assay-lot analyzed on the 96-well plate are shown as “Unknown Wells”, the assay-lot-specific ENC file is missing. The ENC file can be

imported in the next window. Click “OK” to close the summary window and continue.

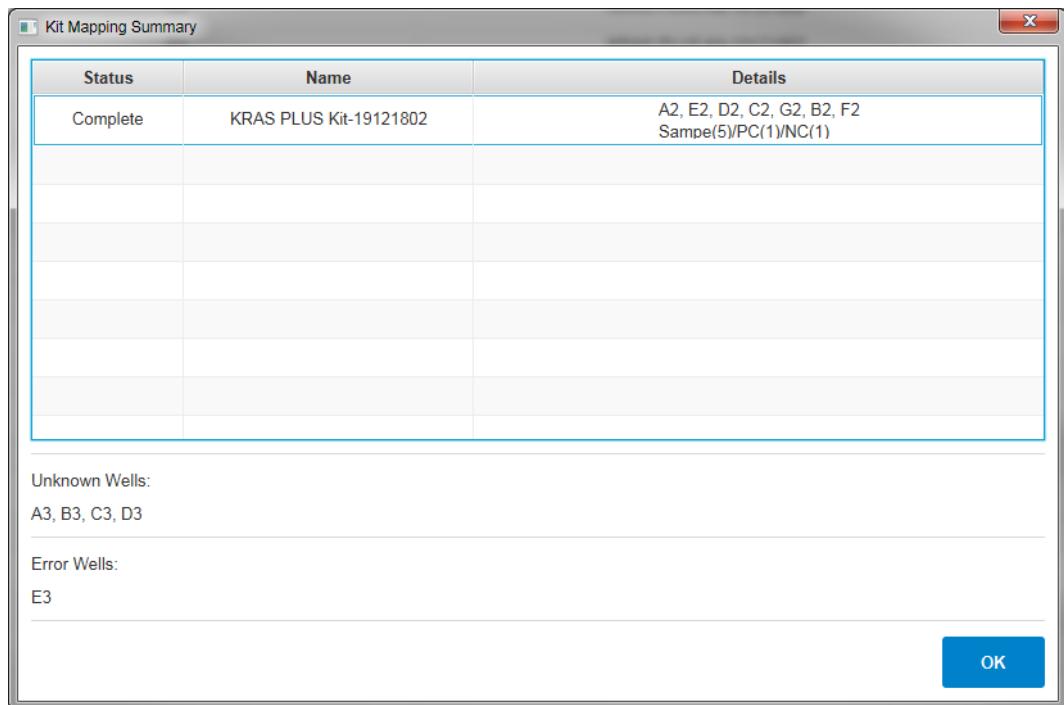


Fig. 28. Kit Mapping Summary

- Step 4. The “Kit Confirmation” window (Fig. 29) shows up after the “Kit Mapping Summary” is closed and lists all Specimen IDs with associated well position, Kit Name and Lot Number.
- Import all missing assay-lot-specific ENC file(s) before confirming the assay by clicking “Import ENC File” (ENC files can be downloaded at: <https://www.plexbio.com/downloads>). After importing the ENC file, all Specimen IDs matching the information of the imported assay-lot-specific ENC file will have their Kit Name and Lot Number updated accordingly.
- Specimen IDs that remain with Kit Name “UNKNOWN” (despite having all ENC files imported, see “note” below) or “ERROR” can manually be associated to any Kit Name/ Lot Number that was identified for any of the other wells in the file (Fig. 29, a and b). Specimen IDs that are manually associated with a Kit Name/ Lot Number will be included in the selected assay-lot specific report (but will not have a result).
- Specimen IDs that are not manually associated with one specific assay-lot but confirmed as “UNKNOWN” will be included in all assay reports associated with this file. Any Specimen IDs confirmed as “Error” will not present in any report associated with this file.
- Ensure the kit-lot information of all wells is correct as the information cannot be changed once the file is confirmed. Click “Cancel” to abort confirming the file. Click “OK” to continue.

Kit Confirmation			
Specimen ID	Wells	Kit Name	Lot Number
S01	A2	KRAS PLUS Kit-19121802	19121802
S02	B2	KRAS PLUS Kit-19121802	19121802
S03	C2	KRAS PLUS Kit-19121802	19121802
S04	D2	KRAS PLUS Kit-19121802	19121802
S05	E2	KRAS PLUS Kit-19121802	19121802
S06	A3	a UNKNOWN	
S07	B3	KRAS PLUS Kit-19121802	
S08	C3	UNKNOWN	
S09	D3	UNKNOWN	
S10	E3	b ERROR	
PC1	F2	KRAS PLUS Kit-19121802	19121802
NC1	G2	ERROR	19121802
		UNKNOWN	

OK Cancel Import Enc File

Fig. 29. Kit Confirmation window

- Step 5. After clicking “OK” in the Kit Confirmation window, a post kit mapping summary window shows up (Fig. 30). Confirm that all wells are associated with their respective kits/ lots. Click “Cancel” to abort the process. Click “OK” to confirm the file to set the status to *FINISHED*. Once the Parental File has the status *FINISHED*, the report(s) will be associated with the Offspring File(s). Note that the report can only be accessed if the assay-specific APP is installed.

Kit Mapping Confirmation		
Status	Name	Details
Complete	KRAS PLUS Kit-19121802	A2, E2, A3, E3, D2, D3, C2, G2, C3, B2, F2, B3 Sampe(10)/PC(1)/NC(1)
Unknown Wells:		
Error Wells:		
OK Cancel		

Fig. 30. Post-confirmation summary window

Note: A file can be confirmed even if the Kit name is shown as “Unknown” for one or more samples. It is important to highlight that any sample confirmed with unknown kit name will **not** have a result in the report. The most common explanation for “Unknown” Kit Name is that the assay-lot specific ENC file is missing. Import the assay-lot specific ENC file and DeXipher will automatically associate wells according to the ENC file information. **Only confirm files once all associated ENC files are imported and all samples display a Kit name and Lot Number;** DeXipher supports reading different lots of the same assay and different assays at the same time, which will require multiple ENC files to be imported.

In some rare cases, one (or more) sample(s) may still show kit name “Unknown” even if the ENC file was imported. This can occur if not enough πCode MicroDiscs are in the well for automated assay recognition. Possible causes are explained below. Samples (wells) with insufficient πCode MicroDiscs will not provide results in the report. Confirm the file to access the results of all samples with recognized assay-lot.

The likeliest reason for low πCode MicroDiscs count interfering with automated assay recognition is that not enough πCode MicroDiscs were pipetted into the well. Always vortex the πCode MicroDiscs stock tube and only pipette up to 4 wells before mixing the tube by vortexing again. Problems during sample processing with the IntelliPlex 1000 πCode Processor may also cause a loss of πCode MicroDiscs. Ensure to always use freshly prepared 1x Wash Buffer. Ensure that all bottles and SAPE fill level are as required. Ensure maintenance is performed as required. If the problem persists, contact PlexBio Co., Ltd. or your local distributors.

The assay-specific APP does not need to be installed to confirm a file. However, reports will only be accessible once the assay specific APP is installed, which can be done after a file is confirmed.

Chapter 9. History

The **History tab** is available in all DeXipher license types.

It is important to understand the file status system used in DeXipher before using the function provided in the **History tab** (section 9.1).

The **History tab** provides access to saved files. Available options depend on the file status. An overview is shown in the scheme below:

FILE STATUS				
	EDIT_COMPLETED	ASSAYING	ASSAYED	FINISHED
Discard an existing file	✓			
Add/Change well	✓			
Add/Change well type	✓			
Edit sample information	✓	✓	✓	
View well image		✓	✓	✓
View/Export the raw data of a single well		✓	✓	✓
View/Export the raw data of all wells			✓	✓
View/Export the report				✓
Use other tabs	✓	✓	✓	✓

Created files that are not analyzed by the PlexBio 100 Fluorescent Analyzer can be selected to be analyzed at any time, using the “Resume” option. The well selection and type of files that have not been analyzed can still be edited. Files that have not been analyzed can also be renamed or deleted (deleted file will be removed from the **History tab**).

The results of a file analyzed by the PlexBio™ 100 Fluorescent Analyzer can be accessed. Results can be exported as an XLSX file to facilitate easy and convenient manual analysis, using the “Data” option (section 9.2).

The **History tab** offers the option to analyze results by applying different protocols. Protocols can only be applied to files that are confirmed (Status: *FINISHED*). See section 9.3 for more information on how to apply protocols.

The **History tab** provides access to reports for IntelliPlex-branded assays (DeXipher RUO) and custom assays with applied protocol. Reports are only available for confirmed files (Status: *FINISHED*). Reports in DeXipher RUO are for Research Use Only. DeXipher Basic does not support reports. See section 9.4 for more information.

The **History tab** supports transferring files between different copies of the DeXipher client software using the Export/Import features. See section 9.5 for more information. For more information on changing the permission to access a file, refer to section 9.6.

9.1 File System and Status

It is important to understand the file system and file status.

File system:

The file system consists of two types of files, “Parental Files” and “Offspring Files”.

Any file that is created in the **IntelliPlex Assay** or **Custom Assay tab** is saved to the table in the **History tab** as a file of type *Raw Data* and is defined as Parental File (#=0). The Parental File contains all the well and sample specific information and, once analyzed with the PlexBio 100 Fluorescent Analyzer, all πCode MicroDisc related raw data. Parental Files only contain raw data and do not provide analyzed results or reports. Protocols are applied to *FINISHED* Parental Files to create Offspring Files.

Offspring Files (#≥ 1) are derived from Parental File by applying a custom protocol or APP-specific rules to the raw data saved in the Parental File. Offspring Files are always associated to their respective Parental Files and can be accessed by double clicking the Parental Files or by clicking the ► symbol next to the Parental Files. All protocol or APP-specific results are associated with the Offspring Files, never with the Parental File.

For Parental Files that are created in the **Custom Assay Quantitative** or **Qualitative tab**, DeXipher will automatically create an offspring file (# = 1) based on the specific rules of the applied custom protocol. For Parental Files that are created in the **IntelliPlex Assay tab**, an Offspring File is created once the Parental File is confirmed. The Offspring File will have the APP-specific rules applied. Creating a *Quick Read* files will not automatically create an Offspring File, but custom protocols can be applied to any confirmed Parental File (including *Quick Read* files), which will create an Offspring File based on the specification of the applied protocol.

The availability of results, reports and additional options for each file depends on the status of the file. Note that the status of a Parental File and all its Offspring Files is always identical.

File status:

EDIT_COMPLETED: Parental Files contain the information as entered during setup. The file has not been analyzed by PlexBio 100 Fluorescent Analyzer, and resuming the file still allows editing or deleting the file (section 9.2). As the file has not been analyzed, there are no results associated with a status *EDIT_COMPLETED* file and files cannot be exported. Associated Offspring Files do not contain any data.

ASSAYING:

Parental file is currently being analyzed by the PlexBio 100 Fluorescent Analyzer. Wait until status

changes to *ASSAYED*.

ASSAYED:

Parental files have been analyzed by the PlexBio 100 Fluorescent Analyzer and all πCode MicroDisc related raw data is saved. Results can be exported as an XLSX file containing all πCode MicroDiscs identities and their respective measured fluorescent intensity as well as the median fluorescent intensity of πCode MicroDiscs grouped by identity. Resuming the file allows editing of sample related information such as Specimen ID and Patient ID. For protocols or APP-specific rules to be applied, a Parental File needs to have the status *FINISHED* (section 9.2). Files with the status *ASSAYED* cannot be deleted, do not offer reports, no protocols can be applied, and files cannot be exported.

FINISHED:

Files can no longer be edited. Only parental files with the status *ASSAYED* can be confirmed, which will change the status to *FINISHED* (section 9.2). Changing the status of Parental Files created in the **IntelliPlex Assay** or **Custom Assay Quantitative** or **Qualitative tab** to *FINISHED* will automatically apply the APP-specific rules or custom protocols to the Offspring File (# = 1) and reports are now associated with the Offspring File (section 9.4). Custom protocols can be applied to all *FINISHED* Parental Files, resulting in the creation of the next level Offspring File, which will contain the results and reports based on the applied custom protocol (section 9.3). Only *FINISHED* Parental Files can be exported (section 9.5).

9.2 Saved Files

Any created file can be accessed in the **History tab** (Fig. 31).

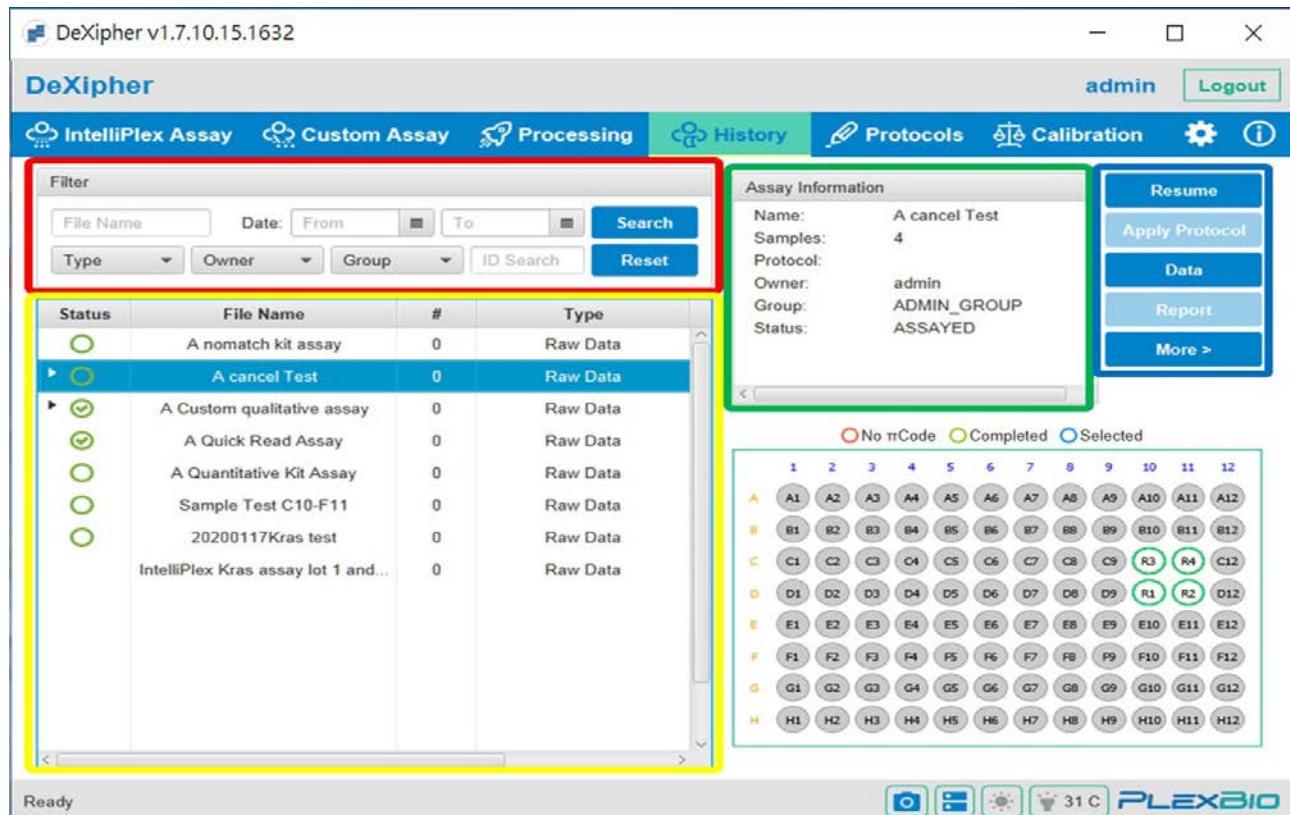


Fig. 31. History tab

Search Assays (Red Box):

Saved assays can be searched by name, keyword, and date or time period the file was created. Results can further be filtered by Type (Raw Data, Qualitative or Quantitative), Owner or Group. Search parameters can be removed using the “Reset” button.

If no search parameters are used, all assays are displayed in the History table (Yellow Box).

History table (Yellow Box):

The History table displays all assays fitting the search and filter conditions.

The table includes the following information for each file:

- File Status (see section 9.1)
- File Name
- # Number (Parental Files are # = 0, Offspring Files are numbered in order of creation)
- File Type (Raw Data, Qualitative or Quantitative Kit, Qualitative or Quantitative Protocol)
- Created Time

Assays in the list can be sorted by status, file name (numeric and alphabetical ascending (0 to 9; A to Z) or descending (Z to A; 9 to 0)), #, type or by time of creation (newest or oldest first). Note that sorting by # or type will only change the order of offspring files associated to parental file.

The assay status is indicated by a symbol in the table (first column). The status is also shown in the “Assay Information” box (top right, Fig. 31: green box):

- **No icon:** The file status is *EDIT_COMPLETED* or *ASSAYING*.
- **Green circle:** The file status is *ASSAYED*.
- **Green circle with checkmark:** The file status is *FINISHED*.

Click on the file in the table to select it (selected files are highlighted in blue). Well and sample information associated with the selected file is shown in the schematic overview of a 96-well plate on the lower left side of **History tab**. The type of sample (R, U, NC, PC, S, C) is indicated. Wells are color coded: blue =selected (not analyzed), green= completed (analyzed), red =No πCode (analyzed but no πCode identified). The results of a green circled wells can be accessed by double clicking the wells in the schematic 96-well plate. Refer to section 8.2 for more information.

Files that have a ► symbol are Parental Files associated with one or several Offspring Files. To access the Offspring Files, click on the ► symbol or double-click on the file name. All Offspring Files associated to the Parental File will be listed.

Assay Information (Green Box):

The “Assay Information” box summarizes generic file information for the selected file such as file name, number of wells (samples), protocol used (if any), File owner and group and file status.

Assay Functions (Blue Box)

- **Resume**

Only Parental Files can be resumed.

Parental Files with the status *EDIT_COMPLETED* (no icon) contain well and sample information but samples have not been analyzed with the PlexBio 100 Fluorescent Analyzer. To analyze a 96-well plate according to the information saved in the Parental File, select the file in the **History table** (selected file is highlighted in blue) and click “Resume”. DeXipher will automatically load the information from the Parental File and switch to the **Processing tab**. Refer to section 8.1 for further instruction.

Parental Files with the status *ASSAYED* (green circle) have been analyzed with the PlexBio 100 Fluorescent Analyzer and contain well and sample information and πCode MicroDisc results. Resuming the file allows editing sample related information such as Specimen ID and Patient ID (section 8.1) and confirming the results to change the file status to *FINISHED* (section 8.3). Parental Files with the status *FINISHED* (green circle with checkmark) have been analyzed with the PlexBio 100 Fluorescent Analyzer and all information has been confirmed. Resuming the file allows to analyze the results of each well in the Processing tab (refer to section 8.2).

- **Apply Protocol**

Protocols can only be applied to Parental Files with status *FINISHED*.

Refer to section 9.3.

- **Data**

Data is available for files with status *ASSAYED* or *FINISHED*.

Select a file from the History table and click Data button to analyze and export results. A pop-up window will show a table with all the πCode MicroDisc sorted by well (additional sample information is also included in the table). Clicking on any of the πCode MicroDisc in the table will show the distribution of that identity in the well (a picture of the well is shown on the lower left area). Just below the well picture is the “Select Well” option, which allows to select only one well to be displayed in the table of the pop-up window.

The “Export Excel” function exports the results as XLSX file format. The exported XLSX file contains all identified πCode MicroDiscs and their respective measured fluorescent intensity as well as the median fluorescent intensity of πCode MicroDiscs grouped by identity. For Parental Files, the XLSX file does not contain any additional sample information such as Specimen ID and Patient ID. For Offspring Files additional sample information is included, and the results are analyzed based on the applied protocol specifications.

- **Report**

Reports are only available for Offspring Files with the status *FINISHED*.

Refer to section 9.4.

- **Permission**

Refer to section 9.6.

- **Import**

Refer to section 9.5.

- **Export**

Only Parental Files with status *FINISHED* can be exported. To export Offspring File, select the associated Parental File and include the Offspring File during the export procedure. Refer to section 9.5.

9.3 Applying protocols

Only available for DeXipher RUO.

Protocols can only be applied to Parental Files (#= 0) with status *FINISHED* (green checked circle).

The screenshot shows the DeXipher software interface. At the top, there's a navigation bar with tabs for IntelliPlex Assay, Custom Assay, Processing, History (which is highlighted in green), Protocols, Calibration, and settings. On the right, there are user info (admin) and logout buttons. Below the navigation is a filter section with fields for File Name, Date (From/To), Type, Owner, Group, ID Search, and Reset. To the right of the filter is an 'Assay Information' panel showing details: Name: A Quick Read Assay, Samples: 8, Protocol: (empty), Owner: admin, Group: ADMIN_GROUP, Status: FINISHED. Below this is a large table of assays with columns for Status, File Name, #, and Type. Several assays are listed, including 'A nomatch kit assay', 'A cancel Test', and 'A Quick Read Assay' which is highlighted with a green checkmark. To the right of the table is a 12x8 grid of wells labeled A1 through H12. Some wells have small circles next to them, indicating status: R1, R5, R6, R7, R8, and R12 have green circles, while others like A1-A4, B1-B4, C1-C4, D1-D4, E1-E4, F1-F4, G1-G4, and H1-H4 have grey circles. At the bottom left is a 'Ready' status message, and at the bottom right are icons for camera, barcode, and 30°C, along with the 'PLEXBio' logo.

Fig. 32. Apply Protocol

Applying a protocol to Parental File will create an Offspring File (#> 0) which will contain the results according to the protocol. Results can be accessed by selecting the Offspring File in the History table and either using the Data button (to download data as XLSX file) or the Report button to access the report.

Note: Before applying a protocol, it is important to emphasize that protocols have defined requirements and should only be applied to files that fulfill those. Applying protocols to files that do not match the control/standard/πCode MicroDisc criteria defined in the protocol will not provide useful information.

To apply a protocol, select the Parental File in the History table and click the “Apply Protocol” button. The protocol can be selected and applied in the pop-up screen. Refer to chapter 10 on how to create/

import protocols.

Once the protocol is selected and applied, the **Custom Assay tab** will open in the sub-tab (Qualitative or Quantitative) specific to the selected protocol. All wells that are included in the Parental File are shown in the 96-well plate (blue circled). Associate the appropriate *Well Type* for each well. The *Well Type* requirements will depend on the selected protocol. To apply the protocol and to create the Offspring File with the report, click the “Apply” button. DeXipher will automatically switch back to the **History tab** and the new Offspring File is available (refresh the History table by clicking “Search”). Select the Offspring File to access the report.

To abort the process of applying a protocol, click “**Abort**”.

Note: Only one protocol can be applied at a time. The parental file may include wells that are not compatible with the selected protocol. Only select the wells in the 96-well plate (in the **Custom Assay tab**) that are associated with the selected protocol. For parental files that include wells associated with different protocols, apply one protocol at a time. For example, apply the first protocol, select and specify all wells associated with this protocol (do not select wells not associated with the protocol) and apply the protocol to create the Offspring File. To apply another protocol, select the Parental File again, select the next protocol and select and specify all wells associated with the second protocol. Apply the protocol to create the Offspring File.

9.4 Reports

Only available for DeXipher RUO.

Reports are only available for Offspring Files with status *FINISHED* (checked green circle; note that the status of Parental File and Offspring File is identical; refer to section 8.3 on how to confirm a file and change the status to *FINISHED*). Reports are created based on the information of the applied protocols or APP-specific rules.

Only Parental File with ► symbol have Offspring File(s); to access Offspring File(s) click the ► symbol or double-click the name field of the Parental File. To access a report, select the Offspring File listed below the Parental File. Then click the “Report” button. The report will automatically open in a new window and can be downloaded as PDF file or XLSX file.

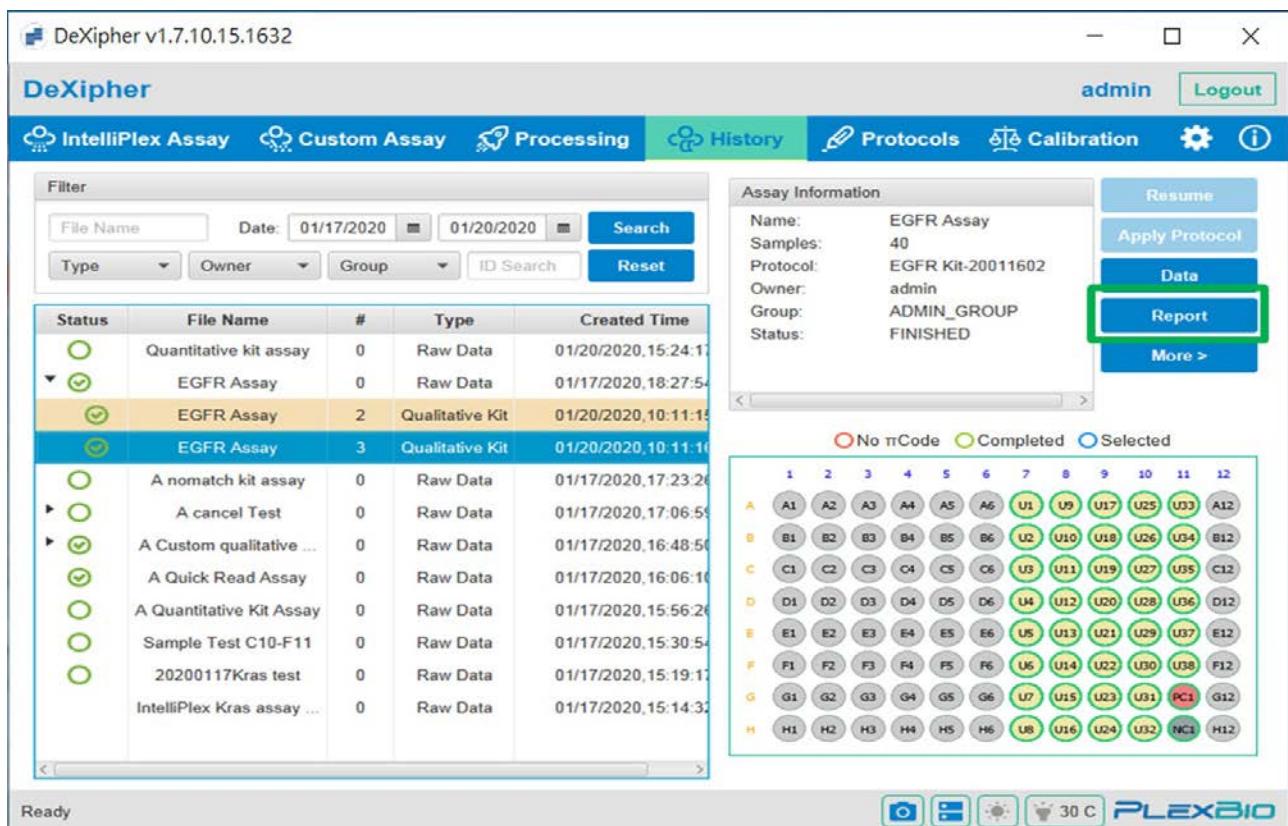


Fig. 33. Generate assay report

Reports for IntelliPlex branded assays will include:

- Report Info:** includes Assay Name, Kit Lot No., Testing Lab Name, Testing Lab Address, and Test Date & Time.
- Summary Data:** includes sample info and results.
- Detailed Report for Each Sample** includes Patient ID, Sample ID, Well position, Time Stamp, Control Results (validity of test) and Results for the Sample.

9.5 Import/Export Files

The **Export** feature (Fig. 34) can be used to backup files externally or to exchange files between copies of the DeXipher client not using a shared server. Only Parental Files with status *FINISHED* offer the Export option. Offspring Files can be added during the export of Parental File.

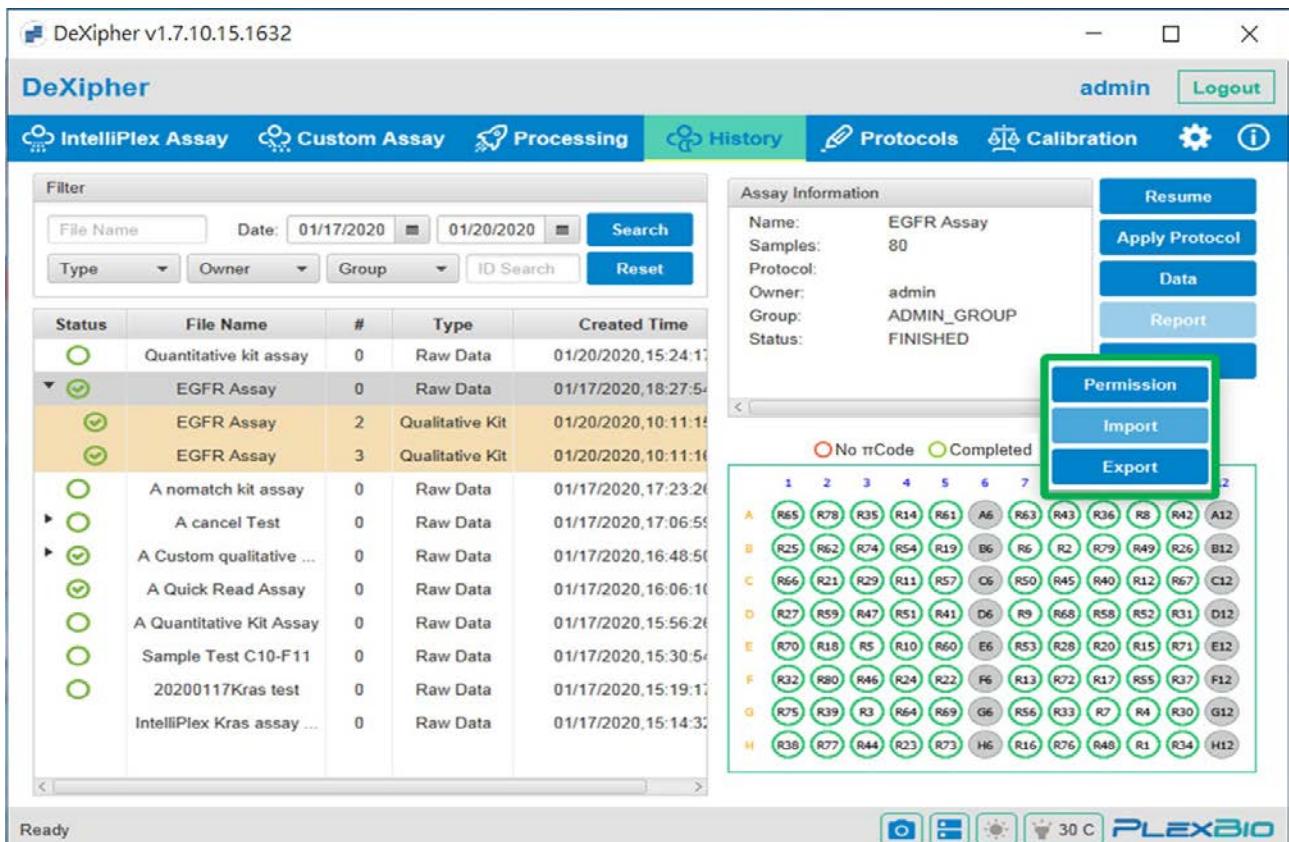


Fig. 34. Permission, Import and Export Functions

To export a file, select the Parental File in the **History table** and click the “Export” button. A pop-up window (Fig. 35) will appear and confirm the file name and creator. Select “Well Images” to include all images into the exported file. Offspring Files can be included by activating the “Protocol” option and selecting the appropriate Offspring Files (defined by # number) from the list. Click “Export” and select a folder to save the file. All exported information will be saved in a ZIP file container that can only be opened by the DeXipher software.

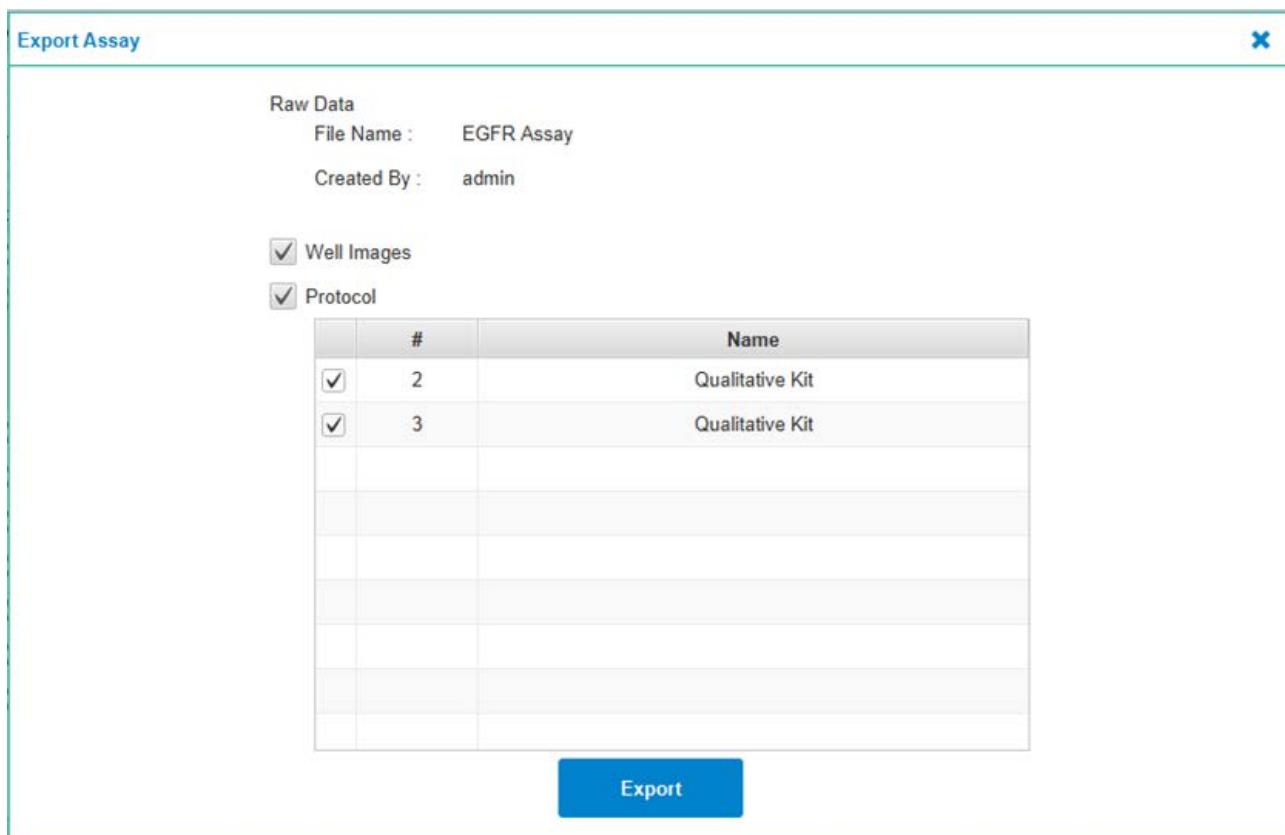


Fig. 35. Dialog window for assay exporting

To import a file, click the “Import” button (Fig. 34) and select the file to be imported. DeXipher will automatically import and add the file to the **History table**. After importing a file, it might be necessary to refresh the **History table** by clicking the “Search” command.

Note: Exported files keep their respective permission settings. Refer to section 9.6 to ensure accessibility of exported/imported files. Remember that not all files are compatible between different license types of DeXipher software. Only files created using Quick Read can be exchanged between all DeXipher license types.

9.6 Permission

To change ownership or permission to a file, select the file in the **History table** and click the “Permission” button.

A pop-up window will appear and allow changing ownership of the file and granting access for one selected group. The default setting is the group which the owner is a member in. All members of the selected group are shown in “Members in Sharing Group”.

Select who has Read Access (owner and/or group) and who has Write Access (owner and/or group). Select either “All” or “Owner” and/or “Group” to give owner and/or group permission.

Click “Apply” to apply changes.

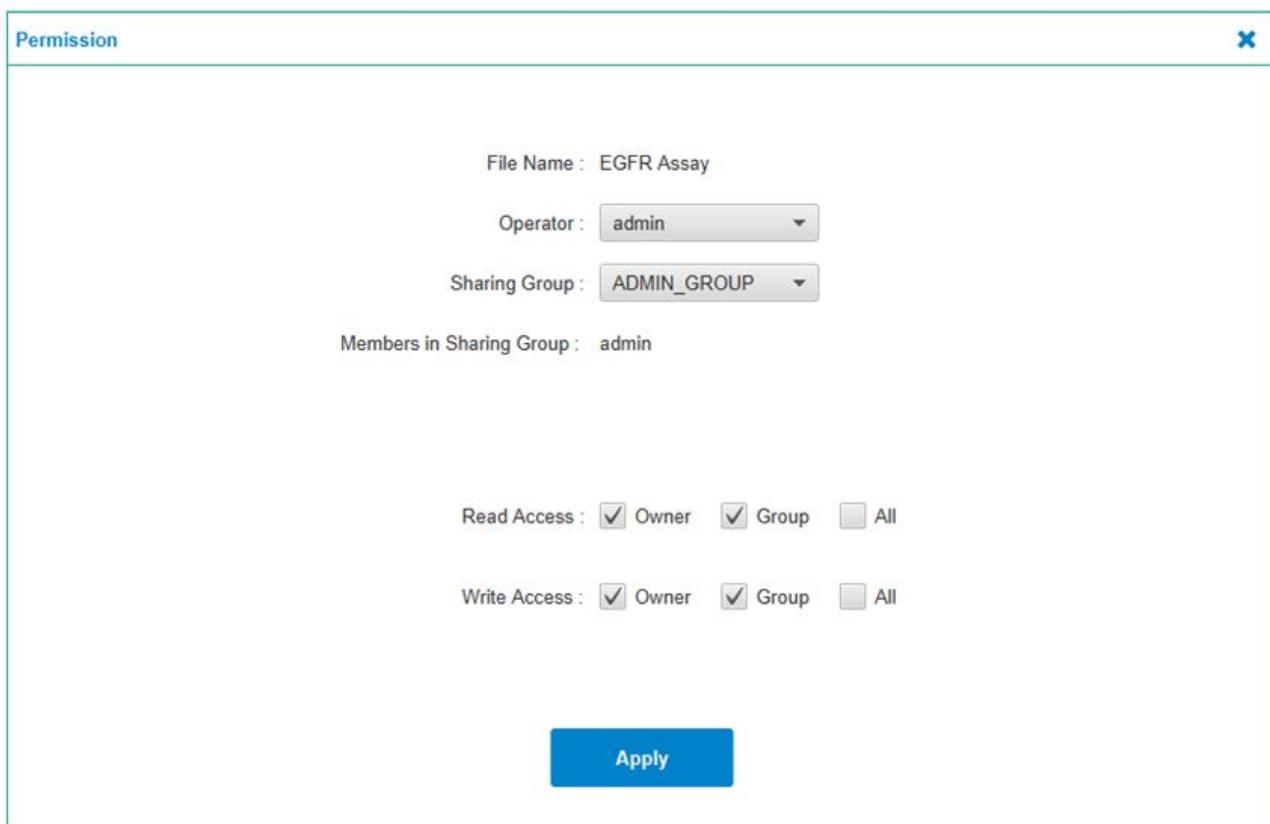


Fig. 36. Dialog window for Permission editing

Note: Ownership and group permission are included in the exported file. Imported files will only be visible to users that are members of the shared group. Refer to section 12.2 for more information on how to manage users and groups.

Chapter 10. Protocol

The **Protocol tab** is only available in DeXipher RUO.

The **Protocol tab** (Fig. 37) includes the *πCode Panel* and the *Configuration* sub-tabs. The *πCode Panel* is used to create the panel of πCode MicroDisc identities used in a protocol and defines which identity is control-, blank- or analyte-specific (section 10.1). The *Configuration* sub-tab allows defining the protocol parameters for Quantitative or Qualitative Assays (section 10.2).

The screenshot shows the DeXipher v1.7.10.15.1632 software interface. The top navigation bar includes tabs for IntelliPlex Assay, Custom Assay, Processing, History, Protocols (which is highlighted in green), Calibration, and a gear icon. On the right, there are admin and Logout buttons. Below the navigation bar, there are two sub-tabs: πCode Panel (selected) and Configuration. The πCode Panel sub-tab contains buttons for Reload, Create, Clone, Modify, Delete, and View. The main content area displays a table of πCode panels:

Panel Name	Status	Description	Created Time
Custom Quantitative Panel 01	Used	Panel for Quantitative assay 01	01/17/2020,17:49:55
Custom Qualitative Panel	Used	Panel for Qualitative assay	01/17/2020,16:18:05
20191115 ALU_20191118193152	Used	from Betty	01/09/2020,17:04:22
Qualitative panel 001	Used	Qualitative panel	01/17/2020,17:35:51
Example Panel		panel for example	01/21/2020,13:42:57

At the bottom, there are icons for camera, barcode, and a 30°C setting, followed by the PLEXBIO logo. A Ready status message is also present.

Fig. 37. Protocol tab

10.1 πCode Panel

The *πCode Panel* sub-tab allows creating a panel of πCode MicroDisc identities used in a protocol. It further allows to associate each identity with specific function: control, blank or analyte/target.

The πCode Panel tab lists all available panels in the table. The table shows the Panel Name, Status, Description and Created Time.

- **Panel Name:** Name of the panel, as given by the User
- **Status:** Status is “Used” if panel is associated with a protocol
- **Description:** Description can be added by User.
- **Created Time:** Time stamp of panel creation.

A menu with all available option is to the left of the table.

- **Reload:** To reload and update the table information.
- **Create:** To create a new panel.
- **Clone:** To clone an existing panel.
- **Modify:** To modify an existing panel. Panels with status “Used” cannot be modified.
- **Delete:** To delete a panel. Panels with status “Used” cannot be deleted.
- **View:** To view an existing panel.
- **Creating a New Panel:** To create a new panel, click “Create”.

Step 1. Enter a unique “Panel Name” (Fig. 38).

Step 2. Optional: Add a description to the panel.

Step 3. Select and add as many πCode MicroDisc identities as needed for the assay.

Step 4. Specify the type for each πCode MicroDisc identity (optional: add description).

Control: This πCode MicroDisc identity serves as control (e.g. fluorescence intensity control).

Blank: This πCode MicroDisc identity serves as blank (e.g. to determine unspecific background). Note that **only one BLANK is supported**.

Analyte: This πCode MicroDisc identity targets an analyte.

Step 5. Save the panel.

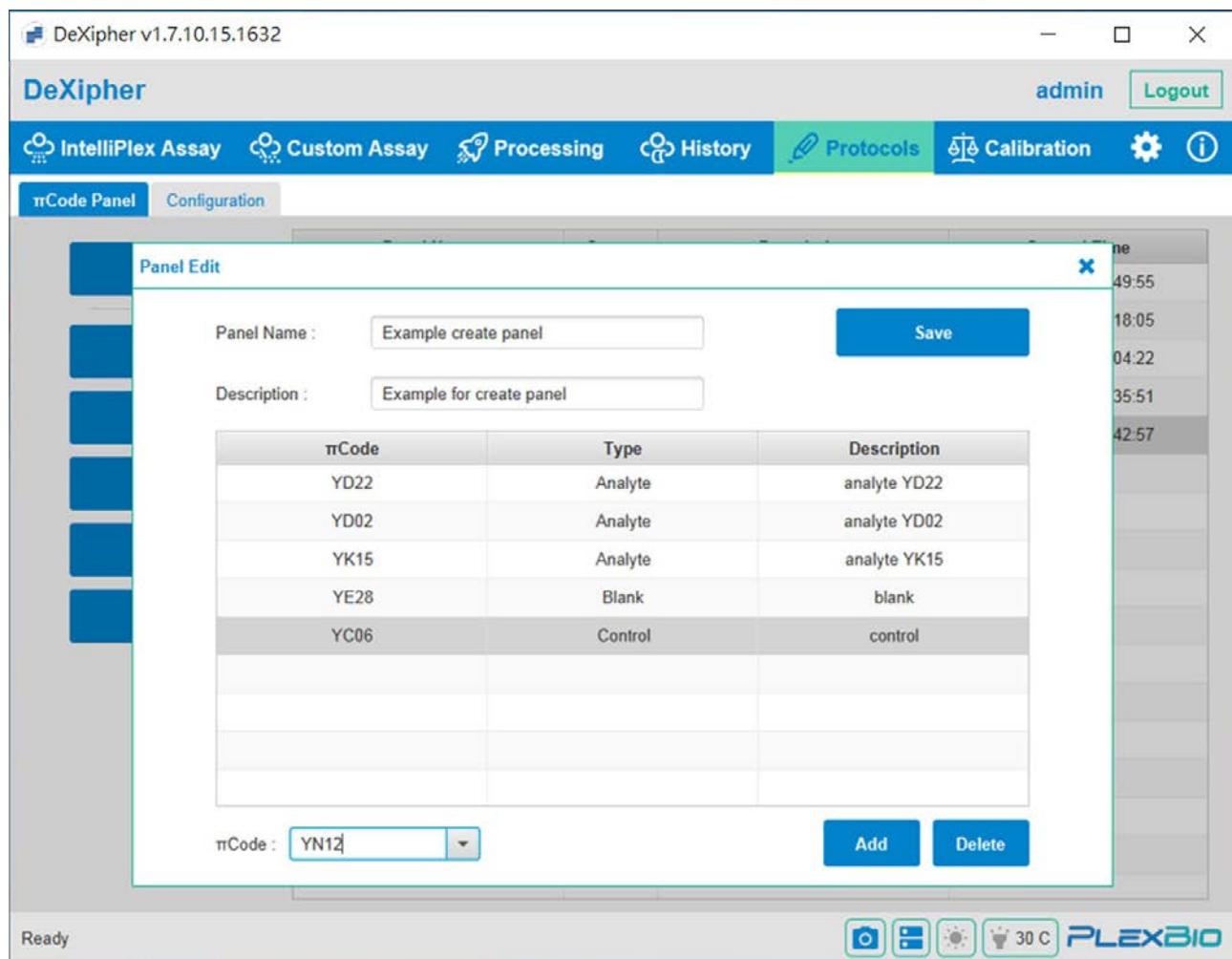


Fig. 38. Create πCode panel

- Cloning a Panel:**

To clone an existing panel, select panel of interest from the table and click “Clone”.

A clone of the original file with all the πCode MicroDisc identities is created. The new file name will be the original name followed by the date the file was cloned (year-month-date-time).

The file name and panel information can be changed using the “Modify” option.

- Modifying a Panel:**

Panels with status “Used” cannot be modified as the information is associated with a protocol, and changing the panel could interfere with the protocol’s functionality. To change the status, the panel must first be disassociated from the protocol (by deleting the protocol or changing the panel associated with the protocol; note: protocols with status “Used” cannot be modified as the protocol is applied to at least one report). Click “Reload” to update all changes to the table.

Select panel and click “Modify” to change/modify the File Name, Description, Add/Delete πCode MicroDisc identities or change the Type or Description of existing πCode MicroDisc identities.

Save changes by clicking “Save” or discard all changes by closing the window using the “X” on the top right edge of the window.

- **Deleting a Panel:**

Panels with status “Used” cannot be deleted as the information is associated with a protocol. Deleting the panel interferes with the protocol’s functionality. To change the status, the panel must first be disassociated from the protocol (by deleting the protocol or changing the panel associated with the protocol; note: protocols with status “Used” cannot be modified as the protocol is applied to at least one report). Click “Reload” to update all changes to the table.

Select panel and click “Delete”.

Ensure only to delete panels that are no longer needed. Panel are permanently deleted and no longer available.

- **Viewing a Panel:**

Any panel can be viewed using the “View” feature.

It is not possible to change file content using the “View” feature.

To close the window, use the “X” on the top right edge of the window.

10.2 πCode Panel Configuration

The *Configuration* sub-tab (Fig. 39) allows to create and manage protocols for quantitative or qualitative assays. New protocols can be created, existing protocols can be cloned, modified, deleted and viewed. Permission for protocols can be set. Protocols can be imported or exported.

All available protocols can be searched and listed in the table. Protocols can be searched by type (Quantitative, Qualitative or use “type” to search for both), Protocol name, Owner and Group.

The table provides the following information for each protocol:

- **Type:** Quantitative or Qualitative
- **Protocol Name:** Name of the protocol
- **Description:** Description can be added (optional).
- **Status:** Status is “Used” if protocol is associated with a file in the History tab
- **Created Time:** Time stamp of protocol creation.
- **Owner:** Owner of protocol
- **Group:** Groups that have access to protocol
- **Permission:** Summary of permission (R = Read; W = Write); see “Permission” below for more details.

Type	Protocol Name	Description	Status	Created Time	Owner	Group
QUANTITATIVE	20191115 ALU_...	from Betty	Used	01/09/2020,17:04:22	admin	ROOT_GROUP
QUALITATIVE	A Qualitative Pro...	A Qualitative protoc...	Used	01/17/2020,16:25:15	admin	ADMIN_GROUP
QUANTITATIVE	Quantitative prot...	Protocol for quantit...	Used	01/17/2020,17:52:18	admin	ADMIN_GROUP
QUALITATIVE	Qulitative protoc...	An Qulitative protocol		01/17/2020,17:37:56	admin	ADMIN_GROUP

Fig. 39. Protocol – Configuration tab

A menu with all available option is to the left of the table.

- Qualitative Assay:** To create a new protocol for a qualitative assay.
 - Quantitative Assay:** To create a new protocol for a quantitative assay.
 - Clone:** To clone an existing protocol.
 - Modify:** To modify an existing protocol. Protocol with status “Used” cannot be modified.
 - Delete:** To delete a protocol. Protocol with status “Used” cannot be deleted.
 - View:** To view an existing protocol.
 - Permission:** To set permission to protocol.
- Import/Export:** To import/export protocols. Note that group permission and ownership will not be exported. Ownership/group permission will be set based on the importing user profile.

Create new protocol for Qualitative Assay:

To create a new protocol for a qualitative assay, click “Qualitative Assay” button.

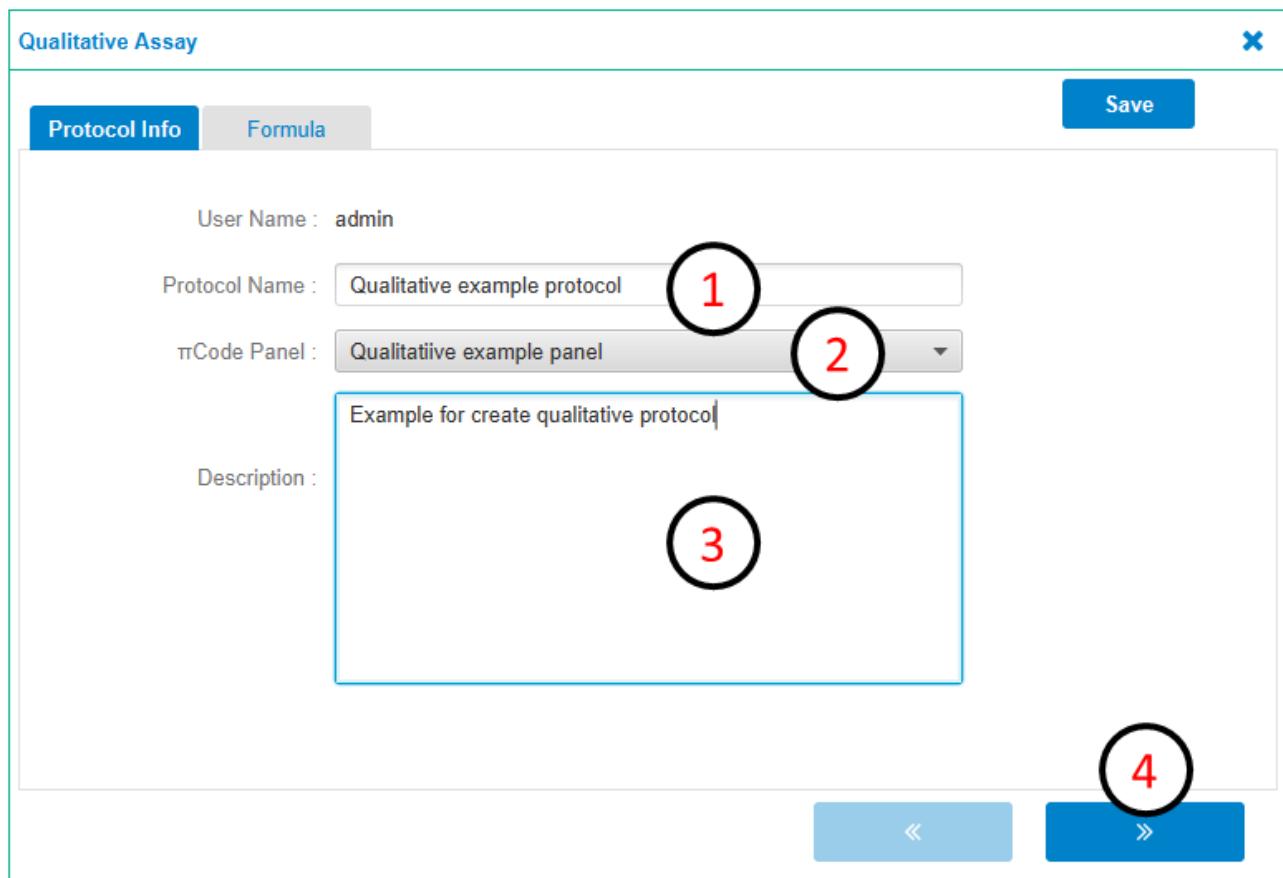


Fig. 40. Protocol – Configuration tab – New Qualitative Assay

Step 1. Enter a unique “Protocol Name” (Fig. 40).

Step 2. Select appropriate πCode panel used with the protocol.

For Qualitative Assays: The MFI of the BLANK determines the fluorescence background of each well and is automatically subtracted from MFIs of all other πCode MicroDiscs.

A protocol can be saved at any time once a Protocol name and πCode panel is selected by clicking “Save”.

Step 3. Optional: Add a description to the protocol.

Step 4. Click “>>” to define protocol requirements in the “Formula” tab of the window.

The screenshot shows the Qualitative Assay software's Formula tab. At the top, there's an 'IF' field containing 'YK02' (circled 1), an operator dropdown set to '>=' (circled 3), and a right-hand side value input field containing '12000' (circled 4). To the right, there are 'Yes:' and 'No:' dropdowns with 'YK02 Pass' and 'YK02 Low' respectively. A note about negative values is visible. Below the input fields is a numeric keypad with standard arithmetic operators (+, -, *, /) and parentheses. A table below the keypad defines conditions: row 1 has '# 1', 'Condition YD11<5000', 'Yes Blank Pass', 'No Blank Failed', and 'Omit negative' checked. Buttons for 'Add' (circled 6) and 'Delete' (circled 7) are at the bottom right, along with navigation arrows. A 'Save' button (circled 8) is in the top right corner.

Fig. 41. Formula tab

- Step 1. Click into the “Expression 1” field to activate (Fig. 41).
- Step 2. Define the left side of your equation. To add a πCode, use “Select πCode” button to select πCode from the panel.
- Step 3. Define the equation (greater >, greater or equal >=, equal =, less <, less or equal <=, not equal !=).
- Step 4. Define the right side of your equation. To add a πCode, use “Select πCode” button to select πCode from the panel.
- Step 5. Define the conclusion of the equation.
Type the statement to show if equation is fulfilled in the “Yes” tab.
Type the statement to show if equation is not fulfilled in the “No” tab.
- Step 6. Click “Add” to add equation to protocol.
- Step 7. Repeat step 1 to 6 for all equations. Use “Delete” to delete selected equation from the protocol.
- Step 8. Click “Save”.

Protocols can be very complex, and the design is assay specific.

Note: If the “Set negative value to 0” box is activated, all negative values will be set to “0” (zero). The protocol shown below (Fig. 42) is an example for illustrational use only. All values are set at random. The protocol is designed to determine which of the two targets has the higher

concentration. Blank and fluorescence controls are included. A πCode panel with four πCode MicroDisc identities is used:

- YA02 – Analyte
- YD03 – Analyte
- YD05 – Control
- YB08 – Blank

The screenshot shows the 'Qualitative Assay' interface with the 'Formula' tab selected. At the top, there are tabs for 'Protocol Info' and 'Formula'. On the right, there is a 'Save' button and a close button (X). The formula builder section has fields for 'IF: Expression 1' and '>= Expression 2'. Below this is a numeric keypad and a set of arithmetic operators (+, -, *, /, (,)). To the right of the keypad are 'Yes:' and 'No:' fields with values 'T' and 'F' respectively. A checkbox labeled 'Set negative values to 0. If the expression is calculated as a negative value, it will be considered zero.' is checked. An 'Add' button is located next to the keypad. Below the formula builder is a table with columns: #, Condition, Yes, No, and Omit negative. The table contains four rows of conditions:

#	Condition	Yes	No	Omit negative
1	YB08<=5000	Background Pass	High background	<input checked="" type="checkbox"/>
2	YD05>=100000	SAPE control pass	SAPE control fail	<input checked="" type="checkbox"/>
3	YA02-YB08>=3000	YA02 signal pass	YA02 low signal	<input checked="" type="checkbox"/>
4	YD03-YB08>YA02-YB08	YD03 expressed	YD03 absent	<input checked="" type="checkbox"/>

On the right side of the table, there is a 'Delete' button. At the bottom of the interface are navigation buttons: '<<', '>>', and a central blue button.

Fig. 42. Formula tab example

Condition 1 – Blank control (YB08):

If $YB08 \leq 5000$ is fulfilled, the statement “Background Pass” will show in the XLSX file or the report. If $YB08 > 5000$, the statement “High background” will show.

Condition 2 – Fluorescence control (YD05):

If $YD05 \geq 100000$ is fulfilled, the statement “SAPE control pass” will show in the XLSX file or the report. If $YD05 < 10000$, the statement “SAPE control fail” will show.

Condition 3 – Analyte (YA02 and YD03):

If $YA02-YB08 \geq 3000$ is fulfilled, the statement “YA02 signal pass” will show in the XLSX file or the report. Otherwise, the statement “YA02 low signal” will show. Note that the “Set negative value to

0" box is activated.

Condition 4 – Analyte (YA02 and YD03):

If $YD03 - YB08 > YA02 - YB08$ is fulfilled, the statement "YD03 expressed" will show in the XLSX file or the report. Otherwise, the statement "YD03 absent" will show. Note that "Set negative value to 0" box is activated. If the sub-equation $YD03 - YB08$ or $YA02 - YB08$ are negative, the value will be set to 0 (zero). That's the statement "YD03 absent" will be shown when YD03 and YA02 are both lower than YB08 even if $YD03 > YA02$.

Create new protocol for Quantitative Assay:

To create a new protocol for a quantitative assay, click "Quantitative Assay" button.

The screenshot shows a software interface for creating a new quantitative assay protocol. At the top, there is a header bar with the title "Quantitative Assay" and a close button (X). Below the header, there are three tabs: "Protocol Info" (which is selected), "Standard", and "Control". On the far right of the header is a blue "Save" button. The main content area has the following fields:

- "User Name : admin"
- "Protocol Name : (circled with red number 1)
- "πCode Panel : (circled with red number 2)
- "Description : (circled with red number 3)

At the bottom right of the content area are two buttons: a light blue "«" button and a dark blue "»" button. Red numbers 1, 2, 3, and 4 are circled around the protocol name input, πCode panel dropdown, description text area, and save button respectively.

Fig. 43. Protocol – Configuration tab – New Quantitative Assay

Step 1. Enter a unique "Protocol Name" (Fig. 43).

Step 2. Select appropriate πCode panel used with the protocol.

A protocol can be saved at any time once a Protocol name and πCode panel is selected by clicking "Save". This will close the setup which can be continued using the Modify function.

Step 3. Optional: Add a description to the protocol.

Step 4. Click “>>” to define protocol requirements in the “Standard” and “Control” tab of the window.

Analyte	YF11	YG09
Unit	ng/ml	ng/ml
Standard-1	36.0	64.0
Standard-2	18.0	32.0
Standard-3	9.0	16.0
Standard-4	4.5	8.0
Standard-5	2.25	4.0
Standard-6	1.125	2.0
Standard-7	0.5625	1.0

Quick Fill

Highest Concentration First Row Last Row
 Initial Concentration: 64
 Dilution Factor: 2
 Concentration Unit: ng/ml
 for all analytes

Apply

Fig. 44. New Quantitative Protocol – Standards

Step 5. Specify the concentrations for each standard.

Each analyte requires the inclusion of a standard series with at least 7 concentration to accurately determine analyte concentration in samples (a standard series with up to 20 concentrations is supported).

The concentration for each standard can be added manually by typing the information into the respective box. If the same dilution factor is used for all concentrations, the “Quick Fill” option on the right side can be utilized by defining the position of the highest concentration (top or last row) followed by entering the highest concentration, the dilution factor and the unit. Click “Apply” to apply to selected πCode MicroDisc standard identity (select “For all analytes” to apply to all πCode MicroDisc standard identities).

Step 6. Select Curve Fitting Method.

Measurements of different standard-concentration for each analyte are used to create an analyte-specific standard curve, which is used to determine the concentration of the respective analyte in the sample. DeXipher supports several algorithms to ensure a standard curve with the best fit, resulting in accurate and reliable quantification of the analyte in the sample.

We recommend that less experienced users select the Best Fit option from the drop-down menu. Experienced users can select a specific curve fitting algorithm based on their application.

Best Fit: DeXipher will determine the best algorithm based on the measured standard data points automatically.

Linear: Using a linear regression to determine the relationship between data points. Most bioassays are often only linear across a specific concentration range.

4PL: Four parameter logistic (4PL) curve regression model is used to follow symmetrical sigmoidal shaped curve. The following equation is applied*:

$$F(x) = b + \frac{a - b}{1 + \left(\frac{x}{c}\right)^d}$$

PL or 5PL v2: Five parameter logistic (5PL) curve regression model is used to follow asymmetrical sigmoidal shaped curve. The difference between 5PL and 5PL v2 is that 5PL v2 extends the focus on the curve. The following equation is applied*:

5PL $F(x) = b + \frac{a - b}{(1 + (\frac{x}{c})^d)^e}$

5PL v2 $F(x) = a + \frac{b}{(1 + (\frac{x}{c})^d)^e}$

*: a = minimum asymptote; b = maximum asymptote; c = inflection point; d = hills' slope; e = asymmetry factor

Note:

1. Each analyte requires the inclusion of a standard series with at least 7 concentrations.
2. Unexpected or extreme data points may lead to suboptimal fitting.
3. Manual selection of curve fitting may lead to suboptimal fitting.
4. A warning for standards with suboptimal coverage is only generated for sample with > 30% error or un-solved data points.

Step 7. Select Weighting Method.

The weighting method determines how each data point affects the standard curve. A weighted standard curve can ensure that the results have a more equal weight in between each level of standards, or have more affection with lower/higher level

standard on the curve fitting. The best weighting method depends on many factors. We recommend that less experienced users select the **Best (Recommended)** option from the drop-down menu. DeXipher will determine the best weighting method based on the measured standard data points automatically.

Available weighting methods are:

- **Best (Recommended)**
- **1/y** = normalize residuals by signal level of each standard respectively to have equivalent weight for all standards
- **1/y²** = emphasize the fitness of lower signal standards
- **None** = emphasize the fitness of higher signal standards

Note: Applying inadequate weighting may result in a suboptimal standard curve.

Step 8. Move to next tab by clicking 

Quantitative Assay

Protocol Info Standard Control Save 

Control Concentration : 2 Reset

Analyte	YF11	YG09
Unit	ng/ml	ng/ml
Control-1	20.0	40.0
Control-2	1.25	2.5

Quick Fill

Highest Concentration First Row Last Row
Initial Concentration: 40
Dilution Factor: 16
Concentration Unit: ng/ml
 for all analytes
Apply

The screenshot shows the 'Control' tab of the Quantitative Assay protocol. A red circle highlights the value '9' in the YF11 unit cell of the control table. Another red circle highlights the 'Save' button at the top right. The 'Protocol Info' and 'Standard' tabs are also visible at the top.

Fig. 45. New Quantitative Protocol – Controls

Step 9. Specify control sample concentration (Fig. 45); control is optional.

Step 10. Click "Save" to save the protocol.

Chapter 11. Calibration

The **Calibration tab** is available in all DeXipher license types.

Before using PlexBio 100 Fluorescent Analyzer for the first time, it is important to calibrate the system. A first calibration will be carried out during the installation of the PlexBio 100 Fluorescent Analyzer by trained service personal.

Calibration once per month is required for the best results. Fail to calibrate once a month results in a non-calibrated warning on the reports.

The following material is required for Calibration:

- IntelliPlex Calibration Kit (Ref. 80035) and lot & serial number specific ENC file.
Note: Each Calibration kit has a unique serial number.
(<https://www.plexbio.com/downloads>; ENC Files section)

Refer to the IFU provided with the IntelliPlex Calibration Kit.

As a reference, a brief overview of the steps required to calibrate the PlexBio 100 Fluorescent Analyzer are listed below:

1. Take out the Calibration Plate and place it in the PlexBio 100 Fluorescent Analyzer.
2. Go to the **Calibration tab** (Fig. 46).

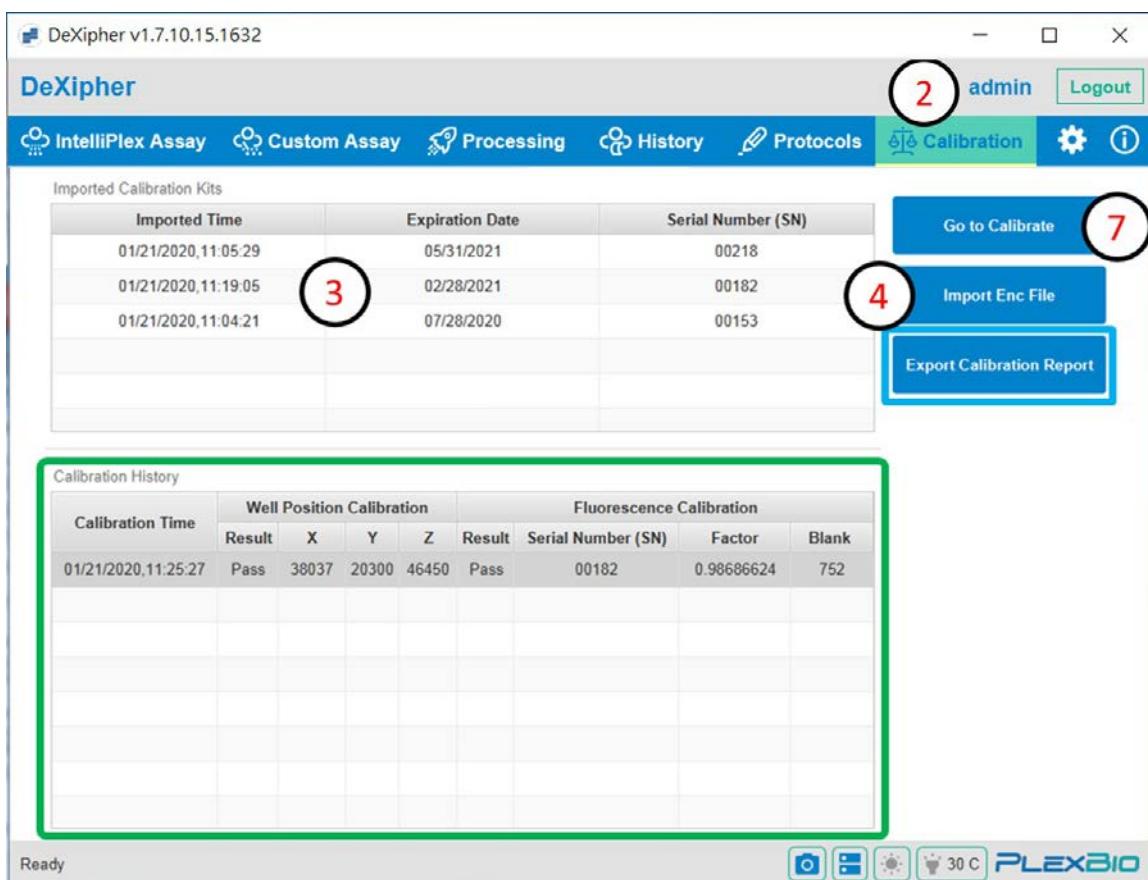


Fig. 46. Calibration tab

3. The “Imported Calibration Kits” table shows a list of all Calibration Kits available in DeXipher.
4. If the Calibration Kit lot is not listed in the table, import the ZIP file containing the ENC files corresponding to the lot and serial number of the Calibration Kit, by clicking “Import ENC File”.
5. Select the ZIP file (which can be downloaded from <https://www.plexbio.com/downloads>; ENC Files section) associated with the IntelliPlex Calibration Kit. The name of the file will be the lot number of the calibration kit.
6. Once the ZIP file containing the ENC files is imported, the calibration kit information is shown in the “Imported Calibration Kits” table.
7. By clicking “Go to Calibrate”, the tab will automatically switch to **Processing tab** (Fig. 47).

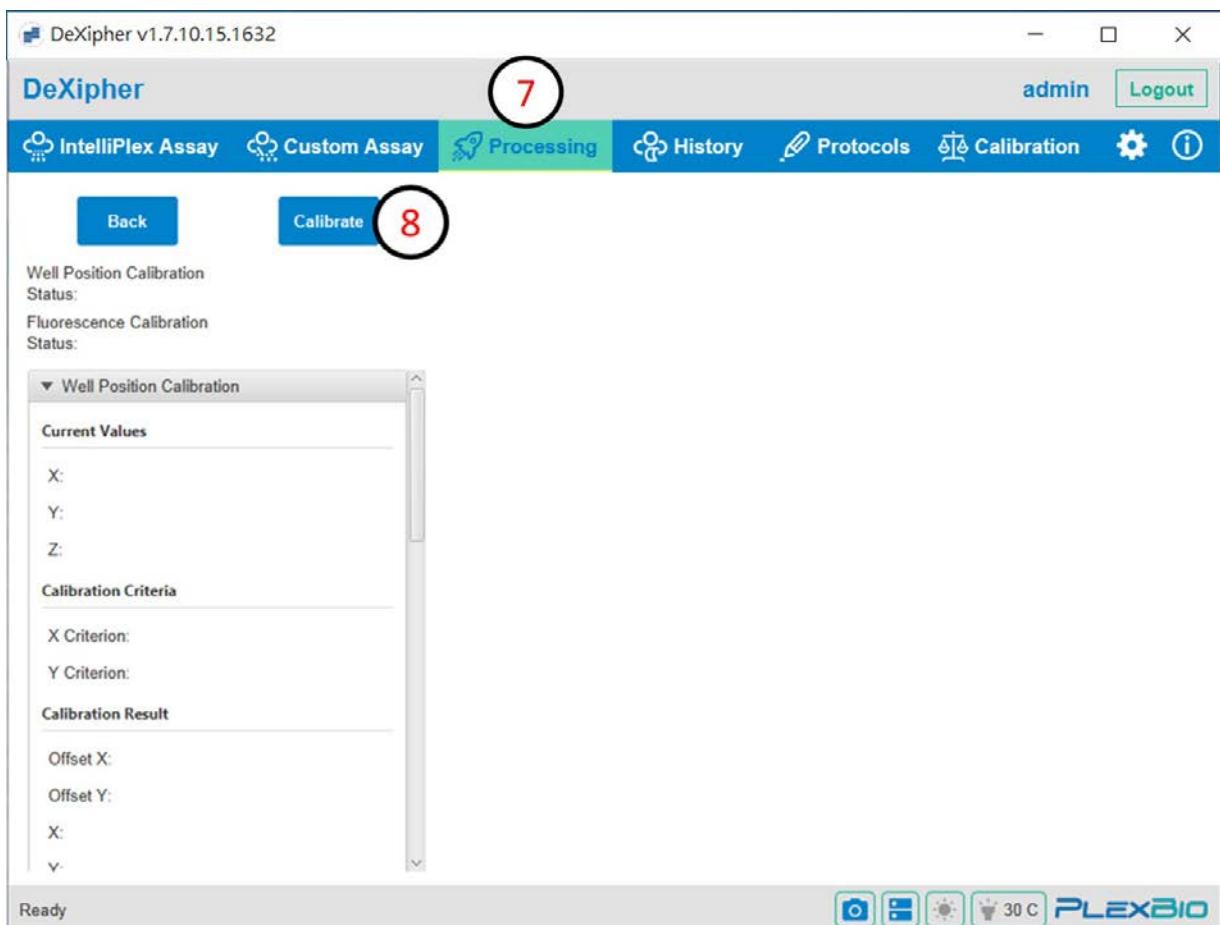


Fig. 47. Processing tab in Calibration

8. Click **Calibrate**.
The system will automatically detect and calibrate the center of the well and the fluorescence signal intensities.
9. Once the calibration is complete, a pop-window will inform whether calibration is successful (Fig. 48).
10. The results of Well Position Calibration and Fluorescence Calibration are shown in the table on the left.

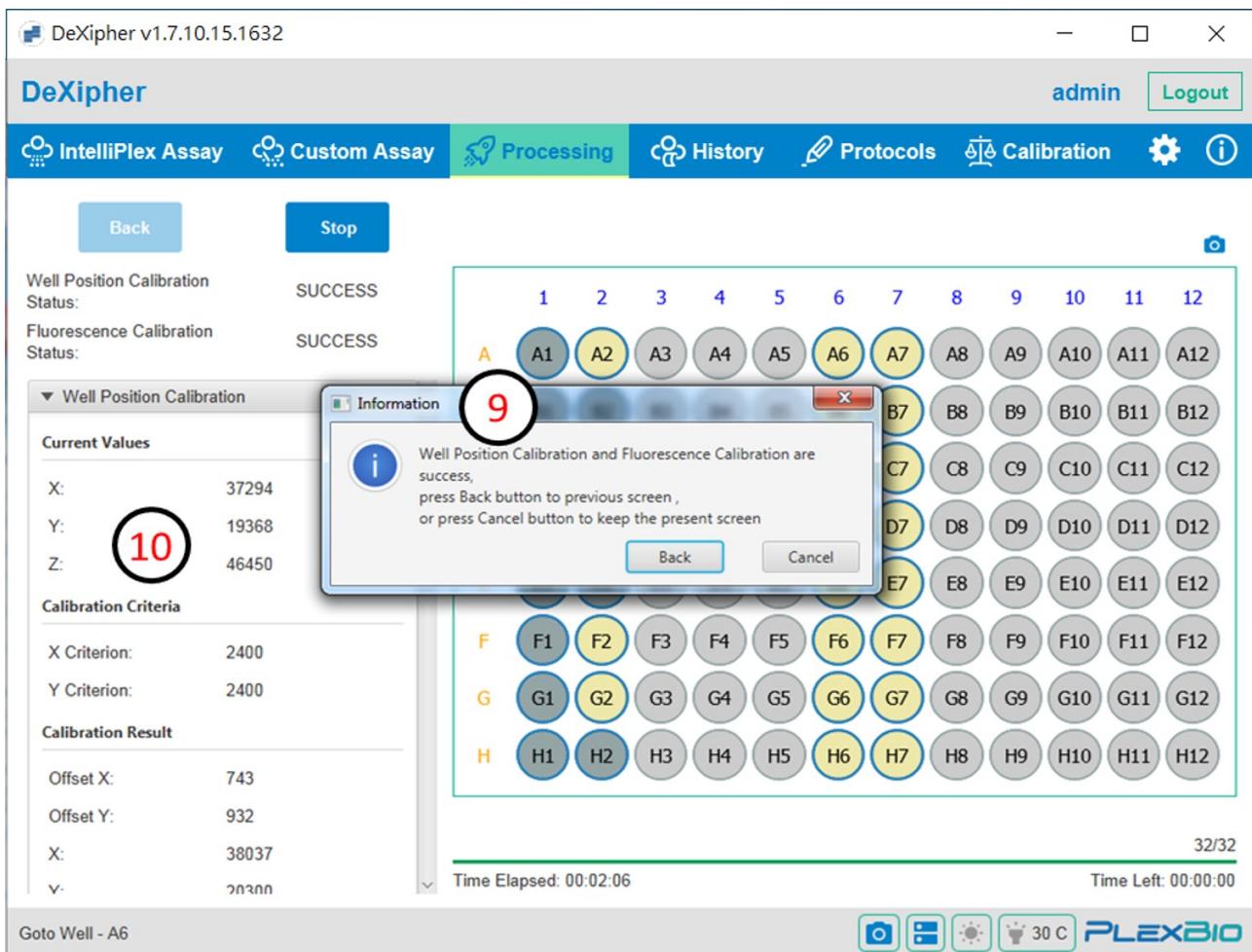


Fig. 48. Calibration process success

The “Calibration History” table in the **Calibration tab** also summarizes the calibration results of each calibration event (Fig. 46, green box).

To Export a calibration report, select the calibration event and click “Export Calibration Report” in the **Calibration tab** (Fig. 46, blue box).

Note: In case calibration is not successful, repeat calibration once. If calibration still fails, contact PlexBio Co. Ltd or your local distributor.

Chapter 12. Settings

The **Settings tab**  is available in all DeXipher license types.

Some sub-tabs are restricted to admins and are not available to all user accounts.

The **Settings tab** allows to change System related settings, create and manage user profiles, import ENC files, and manage ownership of files and protocols.

12.1 System

DeXipher box:

The System tab supports changing the name of the DeXipher client software. The name defines how the DeXipher-client is recognized and displayed in DeXipher server and helps to identify different DeXipher-clients connected to the same DeXipher server.

It allows to change the automated well assignment for multi-well selections. The default setting is “Vertical”: wells will be assigned from A to G. The “Horizontal” option will assign wells from 1 to 12.

Activate Portal Service:

By default, the IntelliPlex Portal is disabled. Enable IntelliPlex Portal if necessary.

DeXipher can connect to the **IntelliPlex Portal**. The **IntelliPlex Portal** is a service offered by PlexBio that provides supporting information for the results obtained by using certain IntelliPlex Assays. For the IntelliPlex Portal to work with IntelliPlex Assay kits, certain sample information is required. Enabling the IntelliPlex Portal will allow to provide sample information during assay setup up (**IntelliPlex Assay tab** or **Custom Assay tab**) or in the **Processing tab** (until the file status is set to **FINISHED**; chapter 8).

Temporary Image File Location box:

The System tab allows to change the temporary image file location and whether temporary images are to be deleted once uploaded to DeXipher-server.

12.2 User Manager

Only available to admins.

DeXipher Basic only allows one active user. This user has admin rights.

DeXipher RUO allow up to active 10 users total by default. If more active users are required, contact PlexBio Ltd., Co or your local distributor.

The User Manager tab allows to create new or modify existing users. The user table displays all

existing users and can be sorted by Login ID, Account Holder, Default Data Group, Role, Data Permission, Status or Created Time:

- **Login ID:** Login ID for each user.
- **Account Holder:** Name of a person/department/company associated with the Login ID.
- **Default Data Group:** Every user is part of a group; users in the same group share files and protocols access.
- **Role:** DeXipher offers three user roles: admin, manager and user.
- **Data Permission:** Shows access allowance of each user.
- **Status:** Active or Inactive.
- **Created Time:** Time stamp of user creation.

Create a new user:

Click “Create” and complete the form to create a new user. The “User Editor” window will open; fill out the form. Click “Cancel” or the “X” (top right) to cancel at any time. Once all information is added, click “Save” to save and create the user.

- **Login ID:** Login ID must be unique for each user. The Login ID is used for DeXipher login.
- **Account Holder:** Name of person/department/company associated with the Login ID.
- **Password:** Password associated with Login ID; used for DeXipher login. The admin can change all users’ passwords. The old password is not required.
- **Role:** DeXipher offers three different roles.
All roles can use **IntelliPlex Assay** (DeXipher RUO only), **Custom Assay** (DeXipher RUO/Basic), **Processing, History, Protocol** (DeXipher RUO only), **Calibration, Settings and Information tab**. The access to functions within the **Settings tab** differs.
- **Admin:** A user with the role “admin” has access to all data; admins can access the User Manager sub-tab in the Settings tab to create and modify users; admins can access the Data Migration sub-tab in the **Settings tab** to transfer ownership.
- **Manager and User:** A user with the role “manager” or “user” has only access to the data (files/protocols) that is part of the user’s group. Access to the data of other groups can be added for each user individually. Neither the role “manager” nor “user” have access to the User Manager or Data Migration sub-tabs of the **Settings tab**.

The default settings between “manager” and “user” are the same. The role “manager” supports the setup and identification of users that act as “manager” and in that role may have permission to more than one group. The role “manager” simplifies identification of users with multi-group access in the User Manager tab.

- **Default Data Group:** Each user belongs to a group. By default, users in the same group share access to files and protocols within this group but can only access other groups data if permission is explicitly granted. If each user is only allowed to access the user’s own data, create a different group for each user. A “supervisor” (=manager) would be granted access to all

- applicable data groups.
- **Default Language:** DeXipher supports Dutch, English, French, German, Greek, Italian, Japanese, Portuguese, Spanish and Simplified Chinese.
- **Data Permission:** Add permission to all or select groups. Note: Users always have access to their own default data group.
- **Status:** Active or Inactive.

Clone:

Select user from the table and click “Clone”. The “User Editor” window will open with the information from the selected user preloaded. The User ID is renamed to the original User ID plus “clone” extension. A new password must be entered.

The clone function simplifies creating new users that only differ in their User ID and Password.

Modify:

All information within a user’s profile can be edited.

The modify function helps to add/remove permission or to activate/inactivate a profile.

The admin can also reset a user’s password without knowledge of the old password.

View:

Shows all information within a user’s profile, but information cannot be edited.

12.3 User

The User tab allows to change settings for the active user.

The active User can change the user’s password (the old password is required).

The active User can change the language of DeXipher User Interface (Dutch, English, French, German, Greek, Italian, Japanese, Portuguese, Spanish and Simplified Chinese).

12.4 ENC

Before using any IntelliPlex-branded assay, users should import the **lot specific ENC file** into DeXipher. Note that IntelliPlex-branded assay specific ENC files can only be imported to DeXipher RUO; DeXipher Basic does not support import of IntelliPlex-branded assay specific ENC files. The lot-specific ENC file can also be imported after an IntelliPlex-branded assay is analyzed but before confirming a file. Reports are only available if the assay-lot-specific ENC file is available at the time the file is confirmed.

Note: DeXipher Basic does not support IntelliPlex Assay. Contact PlexBio Co., Ltd. or your local distributors to obtain DeXipher RUO versions.

Follow instruction below to imported ENC files:

1. Go to **ENC** sub-menu tab in the Settings tab.

All IntelliPlex-branded assay-lot specific ENC files are listed in the table and can be sorted by “Kit Name”, “Lot Number”, “Import Time” or “Expiration Date”. It is not necessary to import a specific ENC file twice.

2. Click “Import” to import ENC files.
3. Select the ENC file obtained from PlexBio official website.

Make sure to import the ENC file that correlates with the lot of the IntelliPlex-branded assay.

The imported ENC file information is now available and displayed in the table.

Note: In case an ENC for an IntelliPlex-branded assay is imported but the supporting APP has not been installed, reports will not be available. Refer to section 6.1 and install the required APP.

12.5 Data Migration

Only available to admins.

The Data Migration sub-tab allows to change ownership of files and protocols from one owner or group to another owner or group. This may become necessary when the status of an owner is no longer active to ensure all files/protocols of this owner remain active and available.

To transfer ownership, select all that applies and click “Transfer”.

Migration Type:

Owner: Transfer files/protocols from a single owner

Group: Transfer files/protocols from a group

Transfer Data from: Select current owner

Transfer Data to: Select new owner

Chapter 13. Information



The **Information tab** summarizes information about the DeXipher software, offers access to log files, summarizes network details, includes a brief description for error codes, and allows to change to DeXipher license type.

About:

The About sub-tab summarizes information related to the local DeXipher-client, including software version and license type.

Log:

The Log sub-tab offers access to all log files. Log files can be searched by type, User Name, action, time, DeXipher Serial Number or name, Instrument (=PlexBio 100 Fluorescent Analyzer) Serial Number.

All results according to the selected filters are displayed in the log file table.

To export a log file, select the file from the table and click “Export Log” on the lower right screen.

Network:

The Network sub-tab allows to see all DeXipher clients and PlexBio 100 Fluorescent Analyzer that are part of the network.

Error Code:

The Error Code sub-tab provides an overview of error codes that DeXipher is using.

License Generate Tool:

The License Generate Tool sub-tab is used to change the license type of the DeXipher software. Contact PlexBio Co., Ltd. or your local distributor for further information.

Chapter 14. Maintenance

14.1 General Maintenance Precautions

Read and follow the general maintenance precautions:

Personnel that use, maintain, or clean the PlexBio 100 Fluorescent Analyzer should be trained in standard laboratory safety practices and should follow those practices when handling the instrument.

※Warning: Biohazard

Samples and waste fluid can contain biohazardous materials. Where exposure to biohazardous material exists, follow appropriate bio-safety procedures, use personal protective equipment, and use ventilation devices.



※Caution

Users should not perform any maintenance or cleaning of the electrical components in the system.

14.2 Daily

- To clean the surface of the PlexBio 100 Fluorescent Analyzer, wipe the surface of the cover or the stage (plate holder) with a soft cloth with mild soap solution or 70% ethanol.
- If the lens requires cleaning. Use lens cleaning air blower or a gentle brush to remove particles first. If there are marks or spots on the lens, wipe the lens with lens tissue using 70% ethanol.
- Shutdown the PlexBio 100 Fluorescent Analyzer after daily use to prolong its lifetime.

14.3 Monthly

Monthly Calibration is recommended. See chapter 11.

14.4 Annually

Annual maintenance is required to perform by a trained specialist. Contact PlexBio Co., Ltd. or local distributors to schedule annual maintenance.

14.5 Backup

It is recommended to backup data regularly.

The following options can be used to create a backup.

Backup of results/reports only:

The results of all files can be exported as XLSX file as described in section 9.2 Saved Files: Resume and Results. All reports can be exported as PDF file as described in section 9.4 Reports.

Backup of exported XLSX and PDF files will contain all results and use the least amount of storage space, but files cannot be imported into DeCipher.

Backup of files:

Files can be exported from the DeCipher software as described in section 9.5 Import/Export file. The exported file contains all the information (including pictures if selected during export) and can be imported to DeCipher.

Windows Image Backup:

We recommend taking advantage of the Image Backup function included in the Windows operating system or to utilize alternative Image Backup software packages. This option will allow to generate a backup of the hard drive including all installed software.

Chapter 15. Troubleshooting

Troubleshooting procedures help users identify and remedy problems with the PlexBio 100 Fluorescent Analyzer. The following table identifies the possible causes of problems or error messages and actions that can be taken. If the solution provided does not correct the problem, or there is no user action stated in the table, contact PlexBio Technical Support for further service.

The following messages may be displayed by Indicator Light:

Indicator Light Status	Cause	Solution/Comment
Flashing in Red	1. Disconnected from PC 2. System warm up failed	1. Check that PC is connected and restart PlexBio 100 system 2. Restart PlexBio 100 system and PC. Do not open the lid during warm up process
Alternating Flashing in Pink and White: reading has stopped before completion.	Lid opened during reading	Do not open the lid during system reading. Close the lid.
Steady Pink	System warming up	Do not open the lid and wait until system warm up is completed (≤ 15 min)

The following messages may be displayed on the Status Bar in the DeXipher software (see section 5.2):

Status Bar Indicator	Cause	Solution/Comment
One or more indicator turns Red/Grey for more than 30 seconds	Camera status error	Check Ethernet cable connection and restart DeXipher
	Stage (plate holder) status error	Check USB cable connection and restart DeXipher
	Bright/Fluorescence status error	Restart PlexBio 100 system and PC
	LED Temperature status error	Restart PlexBio 100 system and PC

The following error in the DeXipher Software:

Problem	Cause	Solution/Comment
Failed to login	1. Incorrect user ID/ password. 2. Server has been stopped 3. Network connection has been blocked.	1. Login with correct user ID and password. Admin can reset all users' passwords. 2. Following 4.2.3 to solve the problem If problem cannot be solved, contact Plexbio or local distributors.
APP Install/Uninstall errors.	The corresponding KIT APP is not installed.	1. Reinstall KIT APP. 2. Referto kit IFU or Contact Plexbio or local distributors for KIT APP installation.
Kit APP not opened with error message "Kit app not found"		
Failed to save panel.	1. Insufficient storage available. 2. SQL server has been stopped.	1. Free up disk space on your computer. 2. Activate SQL server. Contact Plexbio or local distributors.
Failed to import kit	1. ENC file was altered or damaged. 2. SQL server has been stopped.	1. Download the ENC file from website and import again. 2. Activate SQL server. Contact Plexbio or local distributors.
Failed to initiate stage	1. Cable is disconnected or loose. 2. Improper operation or system error.	1. Make sure all cables are well connected. 2. Restart PlexBio 100 system and PC
Failed to export data	1. Insufficient storage available. 2. SQL server has been stopped. 3. System error.	1. Free up disk space on your computer. 2. Activate SQL server. Contact Plexbio or local distributors. 3. Reinstall DeXipher.
Kit APP not responsive	1. Adobe Reader was not installed (10.0 or above). 2. System error.	1. Download and install Adobe Reader from website (10.0 or above). 2. Reinstall KIT APP.

Note:

PC specific problems are not included in these tables. For help with PC problems, contact PlexBio Technical Support or the technical service of your PC manufacturer.

Chapter 16. Contact

PlexBio Co., Ltd.

6F-1, No. 351, Yangguang St., Neihu District
Taipei City 11491, Taiwan

Contact Information

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General info.: marketing@plexbio.com

Technical Support info.: service@plexbio.com

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IntelliPlex™ 1000 πCode Processor

User Manual

PlexBio Co., Ltd.



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- For Emergency Use Authorization Only.
- For *In Vitro* Diagnostic Use Only (IVD).
- For Prescription Use Only (Rx).
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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Chapter 1. Instruction

1.1 Overview

Read this manual carefully before using IntelliPlex™ 1000 πCode Processor. It provides essential information about the following aspects of IntelliPlex 1000 πCode Processor:

- Safety and Regulatory Requirements
- Labeling
- Technical Information
- Installation
- Operation Procedures
- Settings
- Maintenance

1.2 Warnings



When operated in a safe environment according to the instructions in this user manual, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury or possible damage of the instrument. The protection provided by the instrument may be impaired if it is used in a manner not specified in this instruction.

1.3 Symbols

The following symbols may appear throughout this manual with safety warnings, identification, conditions, instructions and regulatory agencies.

Table 1. List of symbols

[Grab your reader's attention with a great quote from the document or use this space to emphasize a key point. To place this text box anywhere on the page, just drag it.]

Chapter 2. Safety and Regulatory Requirements

Please read the safety information in this chapter before using IntelliPlex 1000 πCode Processor. The system contains electrical and mechanical components which may result in injury and damage of the instrument if handled improperly. Do not perform procedures on IntelliPlex 1000 πCode Processor that are not specifically described in the user manual.

2.1 Product Description

IntelliPlex 1000 πCode Processor is a 4-in-1 workstation and is intended to be used with dedicated 96-well plates for sample washing, shaking, incubation and automated fluorescence labeling. The workstation is specifically designed for the πCode™ MicroDisch hybridization, washing and labeling steps that precede fluorescent detection by the PlexBio™ 100 Fluorescent Analyzer. Additionally, this workstation can be utilized for ELISA assay washing if carried out in matching 96-well plates.

2.2 Regulatory Labels

The following label appears on the IntelliPlex 1000 πCode Processor. It displays the model, serial number, power requirements, regulatory status, electrical safety certification and manufacturer information.

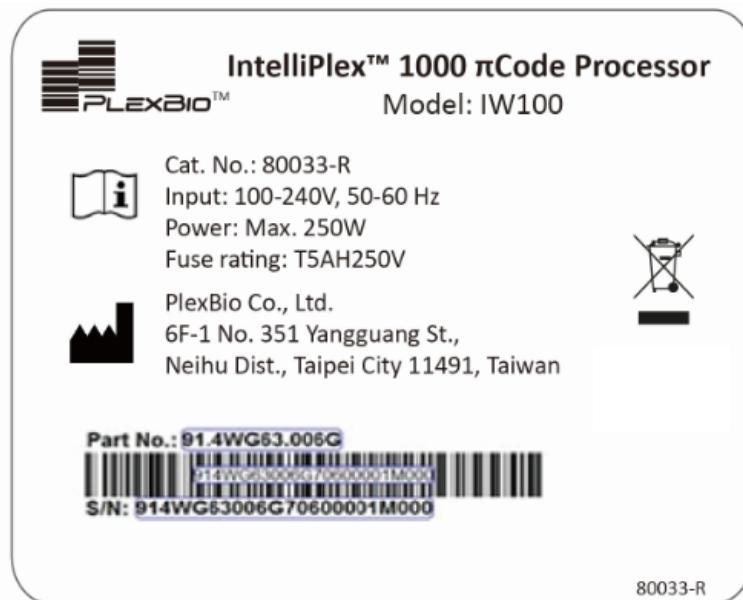


Fig. 1. Label of IntelliPlex 1000 πCode Processor

The following symbols may also appear on the instrument and user manual as necessary.

2.2.1 Testing and Certifications

The IntelliPlex 1000 πCode Processor has been tested and complies with the safety requirements.



Conforms to UL STD. 61010-1,
61010-2-010 and 61010-2-101;
Certified to CSA STD. C22.2 NO. 61010-1,
61010-2-010 and 61010-2-101

(ETL mark)



(FCC mark)

2.2.2 Safety Precautions

The following symbols indicated that safety precautions should be taken before and during system operation.



General Cautions

This message is used to indicate general caution.

- Please handle with extreme care when moving or relocating system as mishandling may damage the system.
- Lift the unit from the base only. **DO NOT** lift by or put excessive force on the plastic case.
- **DO NOT** put excessive force on the stage or it may become tilted or bent. This will severely interfere with the performance of the system.



Electrical Cautions

This message is provided to avoid electric shock or possible damage to the system.

- **DO NOT** open or remove the covers on the system peripherals.
- Use only power cords supplied with the system or cords with grounded outlets properly rated for the system. Ensure the power cord is connected to properly grounded AC outlets only.
- Use of surge protector is recommended.
- Users must not perform any maintenance or cleaning of the electrical components in the system.
- If any malfunction happens, please turn off the power button and then disconnect the power cable. The main plug is used as the main disconnection device; avoid positioning the equipment so that it is difficult to unplug.



**Pinch
Point**

- The IntelliPlex 1000 πCode Processor has modules that move during operation. To avoid any part of the body being caught by a moving part, please keep hands away from the moving part and do not put your hands under the moving part.



Hot Surface

- Please do not touch the heating plate surface until it has cooled.



Biohazard

- Human and animal samples in the 96-well plate may contain biohazardous, infectious materials. Please use personal protective equipment (PPE) including gloves and laboratory coats. Please handle with extreme care when loading the plate onto the stage to prevent spilling. Referto Chapter 7 for cleaning instruction.



**Waste Electrical
And Electronic
Equipment
(WEEE)**

- Within the European Union, the Waste Electrical and Electronic Equipment Directive 2002/96/EC requires the properly dispose of electrical and electronic equipment when it reaches the end of its lifecycle.

If you are disposing an IntelliPlex 1000 πCode Processor, follow local regulations and requirements.

Chapter 3. IntelliPlex™ 1000 πCode Processor Technical Overview

This chapter introduces and describes the technology, module characteristics and technical specifications of the IntelliPlex 1000 πCode Processor.

3.1 Introduction

IntelliPlex 1000 πCode Processor is an automated workstation specifically designed for πCode MicroDiscs hybridization, washing and labeling steps that precede fluorescent detection by PlexBio 100 Fluorescent Analyzer. The IntelliPlex 1000 πCode Processor minimizes user's hands-on time and supports set up of up to six custom protocols (from hybridization to fluorescence labeling).

Features Include:

- User-friendly interface with embedded processing modes for PlexBio assays or customization.
- Multifunctional, automated operation that combines aspiration, buffer dispensing, shaking, rinsing, heating, with magnetic force application to minimize πCode MicroDiscs loss during washing steps.
- Cross-functional capability for both molecular and immunoassays.
- 7-inch industrial touchscreen panel.

3.2 Main Characteristics

The IntelliPlex 1000 πCode Processor automates the shaking, incubation, washing and fluorescence labeling steps for assays utilizing πCode MicroDiscs. The characteristics are provided as follows:

3.2.1 Shaking and Incubation

This function is designed for incubation of compatible 96-well microplates at different temperatures while shaking.

Features:

- Easy to control time and temperature for operation
- Simultaneous display of set and actual time, and temperature
- Heating and shaking platform for compatible 96-well microplates
- Ready to run built-in PlexBio assay programs

3.2.2 Washing

This function is designed to reduce hands-on time and human error during the πCode MicroDiscs washing process.

Features:

- Combines aspiration, buffer dispensing, shaking, automatic rinsing, and the use of magnetic forces to minimize πCode MicroDiscs loss during washing steps
- Simultaneous display of the current washing cycles and status
- Convenience: easy for operation and maintenance

3.2.3 Fluorescence Labeling

This function is designed to reduce hands-on time for the fluorescence labeling process. For compatibility with the PlexBio 100 Fluorescent Analyzer, use of Streptavidin-Phycoerythrin (SA-PE) conjugates for πCode MicroDiscs fluorescence labeling is recommended.

3.3 System Technical Specification

Model	IW100
PERFORMANCE	
Supported plates	96-well plate (Plexbio; Cat. No. 80025 or Greiner Bio-one; Cat. No. 655101)
Processing time (full plate)	3 ~ 6.5 min (one-time wash)
Shaking	Timing setting range: 1 sec ~ 23hr 59min 59sec Max. rate: 1200rpm
Incubator	Temperature range from RT+5°C to 60°C
Safety	Plate position sensor and door sensor
Temperature accuracy	± 0.5°C
Temperature uniformity	1°C
Shaking accuracy	≤ 10%
Dispensing accuracy	≤ ± 6% (Measured under following conditions: 8-way manifold, 150 µL, PlexBio Wash Buffer)
Dispensing uniformity	≤ 4% CV (Measured under following conditions: 8-way manifold, 150 µL, PlexBio Wash Buffer)
MicroDisc loss	Around ≥ 75% of MicroDiscs retained after whole processes. (Measured under following condition: 500 ~ 1500 discs/well in 150 µL PlexBio Wash Buffer)
Residual volume	≤ 5 µL/well (Measured under following conditions: 8-way manifold, 150 µL, PlexBio Wash Buffer)
OPERATION CONDITIONS	
Temperature/Humidity	18°C ~ 32°C (64°F ~ 90°F)/20 ~ 80% RH, non-condensing
Altitude	Up to 2000 meters (6561 ft) above mean sea level
STORAGE CONDITIONS	
Temperature/Humidity	-10°C ~ 70°C (14°F ~ 158°F)/10 ~ 80% RH non-condensing
PHYSICAL CHARACTERISTICS	
Power	100-240V, 50-60Hz, 250W
Dimensions	Without Bottle Carrier: (W)408mm*(D)540mm*(H)470mm With Bottle Carrier: (W)554mm*(D)540mm*(H)470mm
Weight	38 kg (84 lbs)
Fuse rating	T 5A H 250V
OTHERS	
Pollution degree	2
Method of disposal	Electronic waste

Table 2. System Technical Specification

Chapter 4. Receiving the Instrument

Note that IntelliPlex 1000 πCode Processor must be installed and serviced by PlexBio's qualified service engineers only. Before installation, please make sure the environmental conditions meet the operation requirements:

- Indoor use only
- Operating temperature: 18°C to 32°C (64°F ~ 90°F)
- Operating relative humidity: 20% to 80%, non-condensing
- Operating altitude: up to 2000m (6561 ft.) above mean sea level
- Storage temperature: -10°C to 70°C (14°F ~ 158°F)
- IntelliPlex 1000 πCode Processor needs an operation area of 0.36 square meters and needs to be placed at least 10 centimeters from the wall for components connection and efficient cooling.

4.1 Inspect the Instrument Package

IntelliPlex 1000 πCode Processor arrives in a large, corrugated cardboard overpack on skids.



Fig. 2. The IntelliPlex 1000 πCode Processor package

Inspect the shipping box and packaging for signs of damage. In case of damage, please contact your local distributor or PlexBio Technical Support at +886-2-2627-5878 or service@plexbio.com.

Do not open the box. Please schedule an installation appointment with your local distributor or PlexBio Technical Support.



Cautions

The package of IntelliPlex 1000 πCode Processor may be too heavy to lift. Please lift using adequate equipment. Please handle with care.

4.2 Inbox Accessories

The inbox accessories are listed below. All accessories will be inspected during installation process.

Do not open the box before installation.

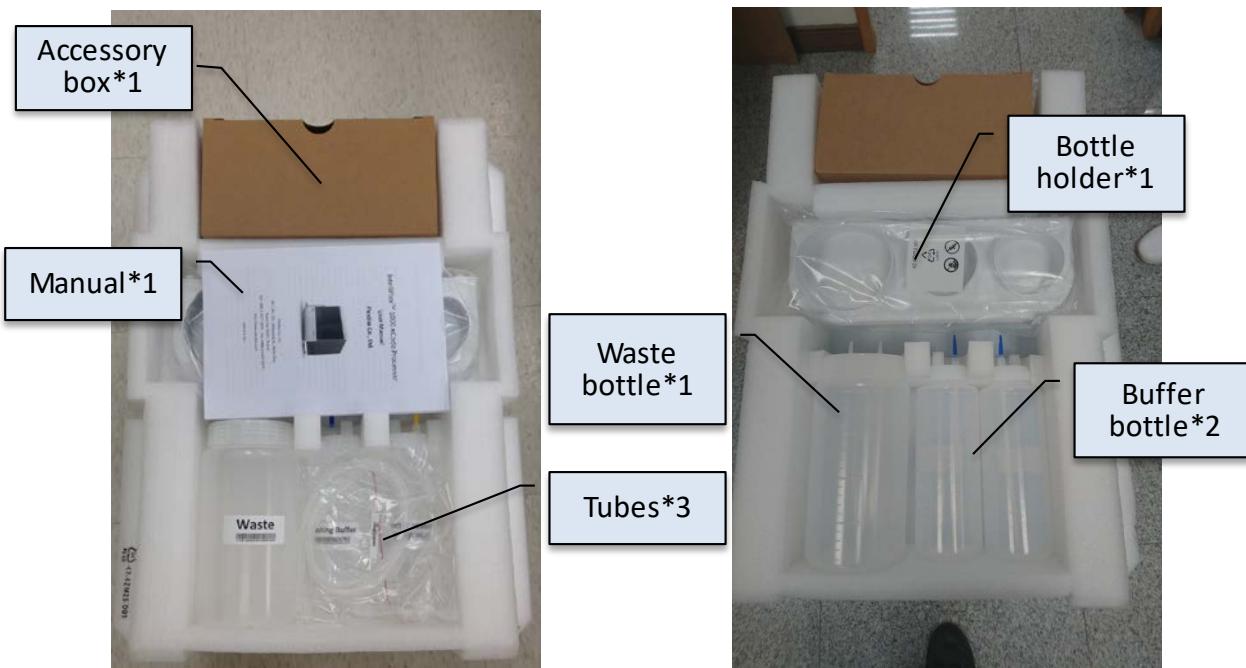


Fig. 3.1 Accessories in Top EPE foam packaging

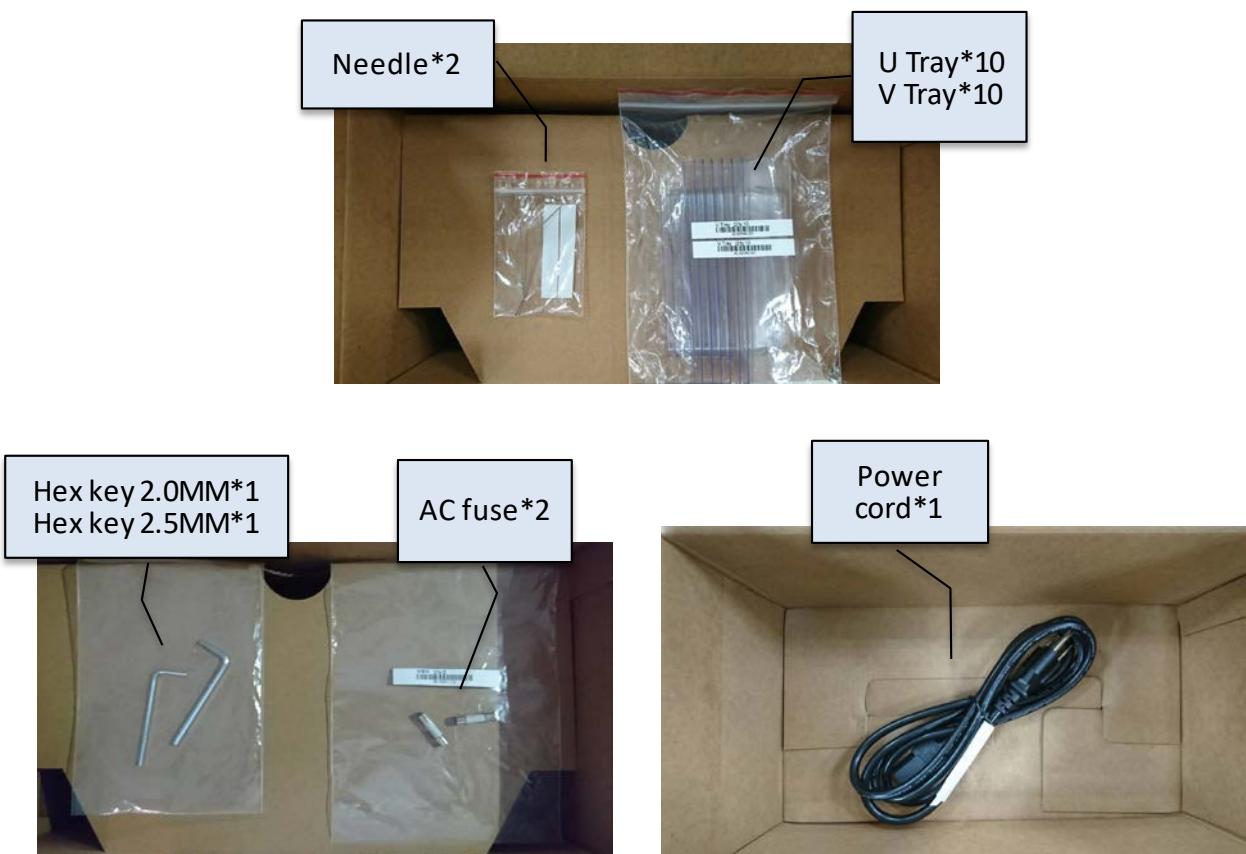


Fig. 3.2 Accessories in the box

Chapter 5. Operation Procedure

Only operate the IntelliPlex 1000 πCode Processor after installation. Do not operate IntelliPlex 1000 πCode Processor if annual maintenance is overdue.

Ensure all daily/weekly/monthly maintenance is performed (refer to Chapter 7. Maintenance for details). Not performing required maintenance will void the warranty. **Moving the IntelliPlex 1000 πCode Processor is not allowed.** If moving is required, please contact Customer Service of PlexBio Co., Ltd. or qualified local distributors.

Before operation, connect the power cord and the corresponding bottles and tubes (Fig. 4). Visually confirm that the U Tray is placed in washing tank (Fig. 5, please also refer to section 6.1.1). Make sure the bottles contain enough ddH₂O and enough wash buffer (refer to section 5.5.2), and the waste bottle is empty.

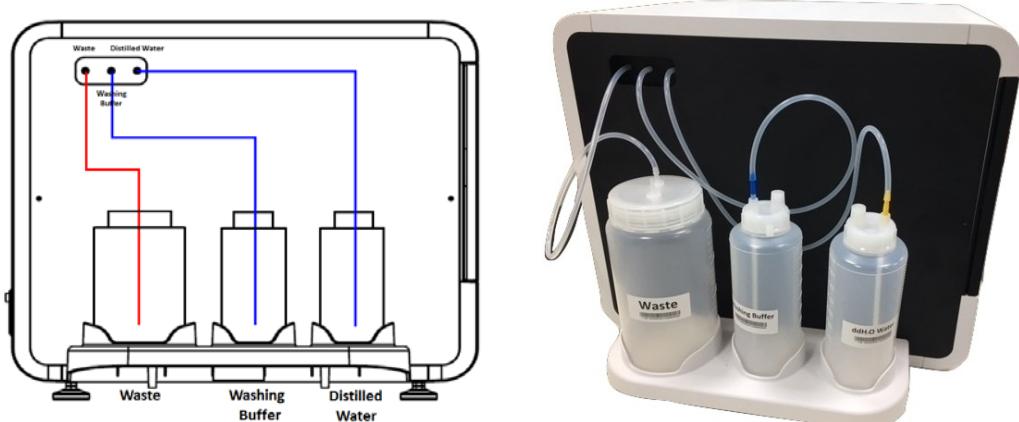


Fig. 4. Connected diagrams

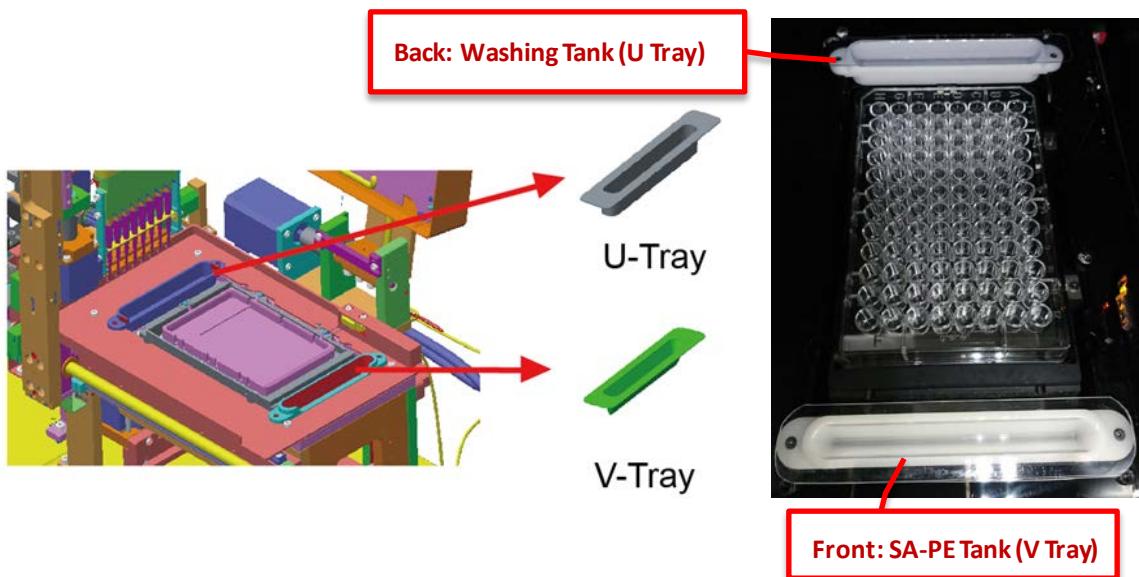


Fig. 5. Tray position

5.1 Startup

Turn on the power at the backside of the instrument. The system will run temperature check and system self-test every time the instrument is powered on. Make sure the ambient temperature is appropriate for instrument operation, the buffer/water bottles, and the waste bottle is empty. Click **OK** to complete **system self-test** process.

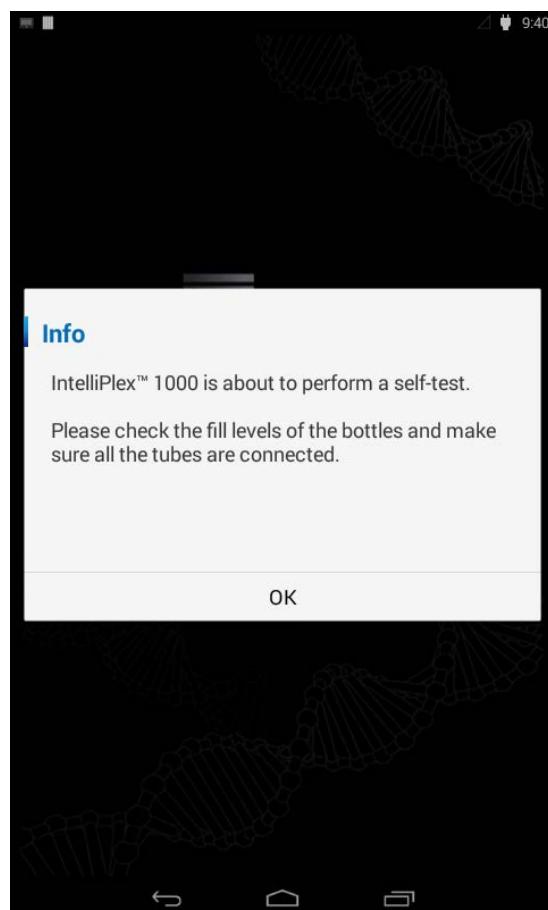


Fig. 6. Interface of system self-test

5.2 Touchscreen Introduction

Figure 7 below depicts the main home screen controls.

(1) Four main function buttons for different assay types.

- Built-in PlexBio Molecular Assay
- Built-in PlexBio Immunoassay
- Built-in PlexBio Applied Science
- Custom Program

(2) Menu button to access settings and maintenance procedures.

(3) Current date and time display.

(4) General information and software versions.



Fig. 7. IntelliPlex 1000 πCode Processor Home Screen

5.3 Predefined Assay Protocols

The IntelliPlex 1000 πCode Processor is installed with predefined assay protocols for the use with PlexBio's assay kits.

5.3.1 RUN the Built-in Assay Kit

For running a PlexBio assay kit, please follow step 1 to 6.

- Step 1. Fig. 8: Select built-in PlexBio assay type (Molecular Assay, Immunoassay or Applied Science).
- Step 2. Fig. 9: Select the desired lanes for processing and click **Next (➔)**.
- Step 3. Fig. 10: Select the corresponding assay to operate.
- Step 4. Fig. 11: Confirm the displayed procedure conditions are correct and click **START**.
- Step 5. Fig. 12: Make sure the pop-up window notices are followed and click **RUN** to start the assay.
- Step 6. Fig. 13: The touchscreen will display real-time operation status of the assay until the run is complete.



Fig. 8. Select the assay types

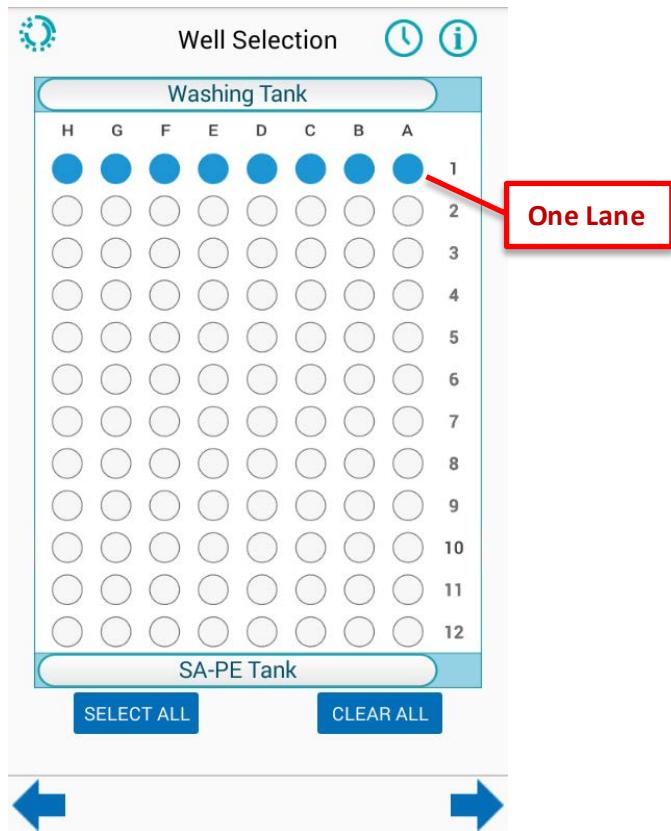


Fig. 9. Select the desired lanes for processing

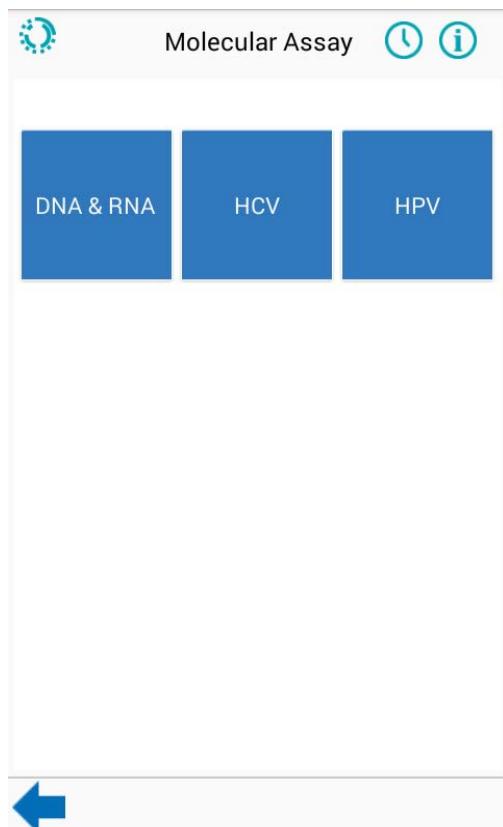


Fig. 10. Select the corresponding assay



Fig. 11. Procedures confirmation

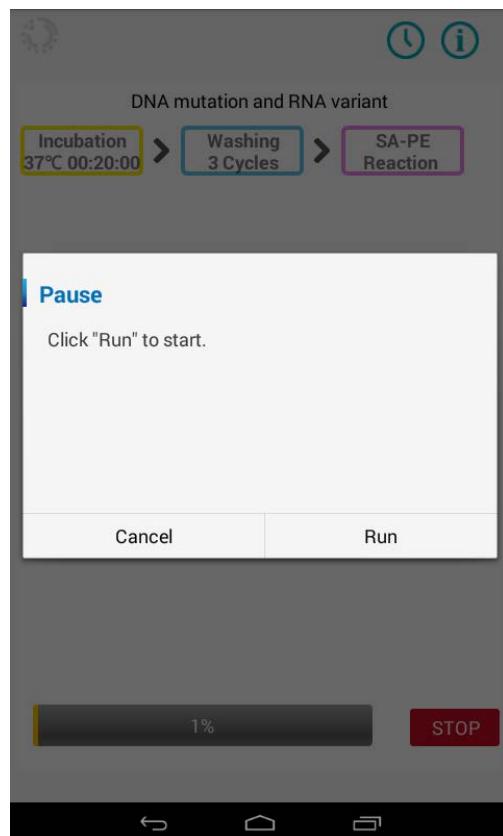


Fig. 12. Pop-up window confirmation

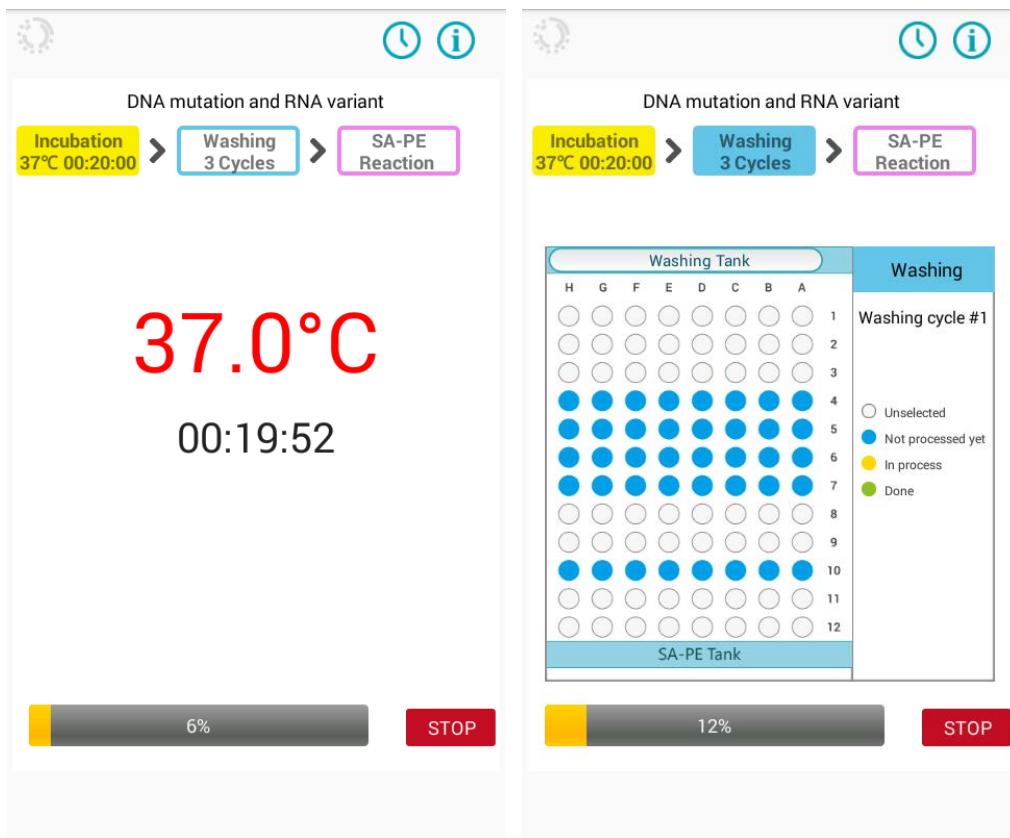


Fig. 13. Real-time display of the assay operation status

5.4 Create a Custom Program

The IntelliPlex 1000 πCode Processor also allows users to create and modify custom assay programs. The following section describes how to setup or edit a custom assay program (a total of six custom programs can be saved).

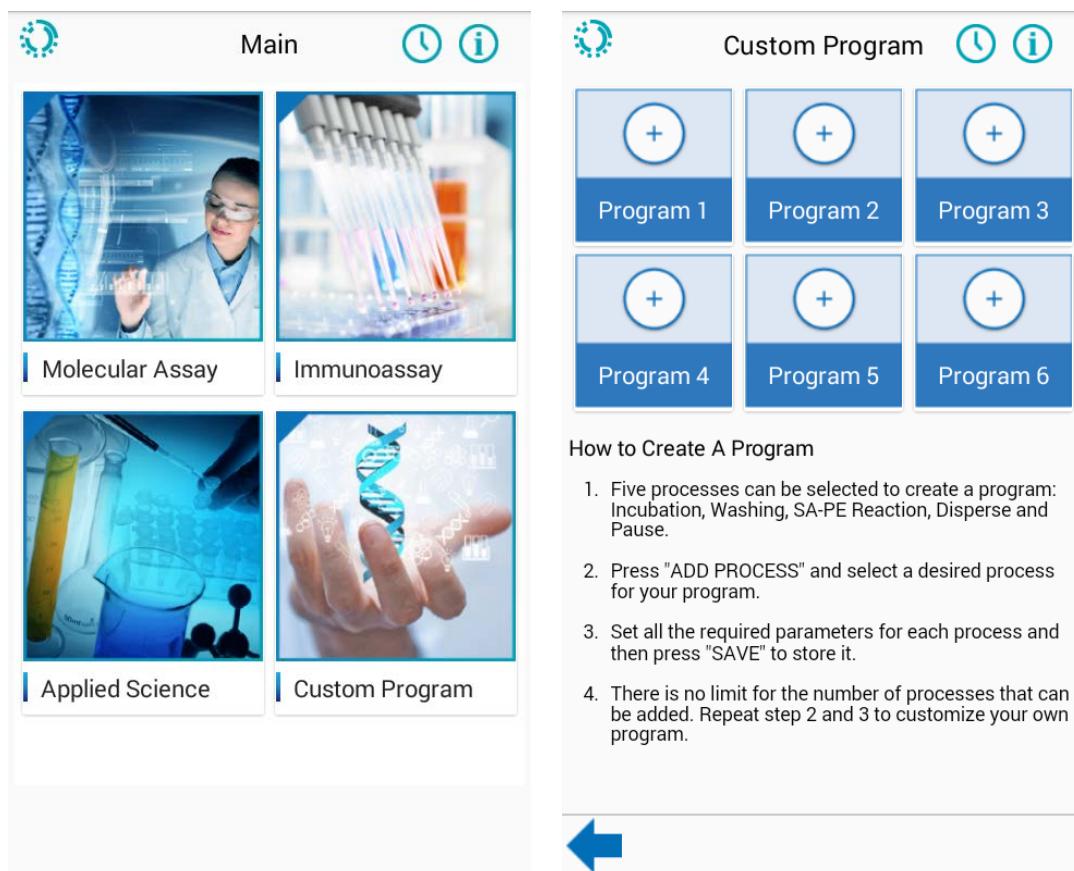


Fig. 14. Interface of custom program

5.4.1 Custom Assay Parameters

Incubation:

- (A) **Heater ON:** Setup the desired incubation temperature by either sliding the setting bar or by keying in the number into the gray box. The offered temperature range is 20°C to 60°C. It is important to highlight that the selected incubation temperature must be 5°C above the current temperature*. Please note that the IntelliPlex 1000 πCode Processor does not have active cooling system. If a new run follows a previous run, please wait until the heating plate has cooled down.

**Current temperature refers to the temperature of the heating plate. Note that the IntelliPlex 1000 πCode Processor measures environmental temperature using the temperature of the heating plate itself. The temperature inside the instrument can be a little higher. Thus, when the incubation temperature is too close to the environmental temperature, error may occur.*

For example, when the current temperature is 30°C, the incubation temperature must be at least 35°C or higher to successfully run the program. If the plate is still warm from the previous incubation process, please wait until the heating plate has cooled down. A pop-up will inform the user of any problems incubating at the selected incubation temperature. Please be aware that custom programs with multiple heating steps in a row may cause termination of the run if the previous temperature was significantly higher than the following temperature.

For example, if a first incubation is carried out at 50°C and immediately followed by a second incubation step at 30°C, an error occurs, and the program will be stopped. Including a wash step between two incubations at different temperatures may be enough to allow passive cool down of the heating plate.

- (B) **Incubation period:** Set up the desired incubation time by directly keying in the numbers into the hour (h), minute (m), and second (s) boxes. The time setting ranges from 1 sec to 23hr 59min 59sec.
- (C) **Close lid during incubation:** This function will decide if the lid is closed during incubation. The lid also includes a heating function. Closing lid during incubation helps in keeping samples more evenly heated. Please note that the lid does not seal the 96-well plate, thus does not prevent evaporation at elevated incubation temperatures.
- (D) **Shake before reaching temp.:** If activated, heating plate shaking and incubation time countdown start once the program has started, independent of the status of the incubation

temperature. If not activated, shaking and incubation time countdown will start once the incubation temperature has been reached.

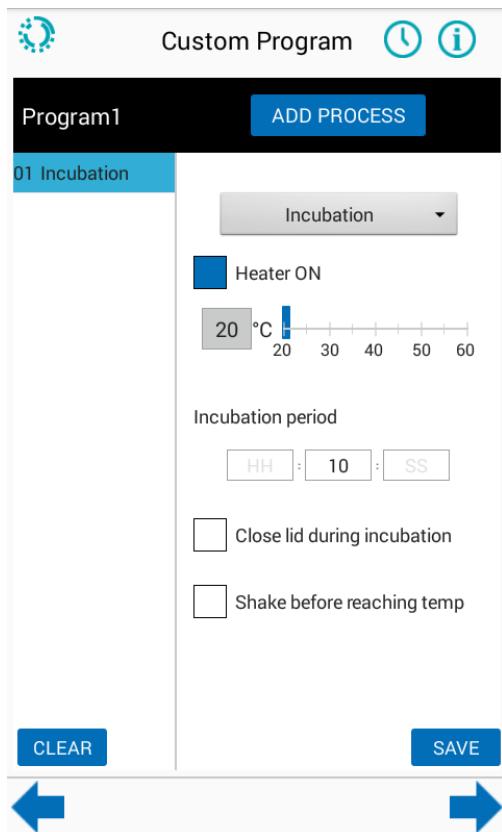


Fig. 15. Parameters of custom incubation procedure

Washing: Users can setup various conditions listed below during the washing process (Fig. 16).

- (A) **Number of Washing Cycles:** Key in the desired number of washing cycles.
- (B) **Washing Volume:** Users can either select the 150 µL washing volume (used for all πCode applications) or the ELISA wash function (which will dispense 200 µL wash buffer).
- (C) **Last Dispense Volume:** If the function is activated, additional 150 µL of wash buffer will be dispensed into each well at the end of the wash cycles followed by 10 sec shaking to spread out/disperse the πCode evenly. If the function is not activated, no liquid will be left in wells after washing process. It is recommended to only activate this function if the wash step ends the program and the plate is subsequently analyzed by the PlexBio 100 Fluorescence Analyzer.
- (D) **Magnetic:** Must be activated for all πCode assays. The magnet will collect and retain πCode at the bottom of the well and only the liquid waste will be aspirated during washing process. The magnetic function is not needed for ELISA assays.
- (E) **Clean Tip:** To prevent any carryover contamination between samples placed in different wells, click on this function and the tips will be cleaned before approaching to next set of wells. The tip cleaning will only be performed at the 1st cycle of each washing process.

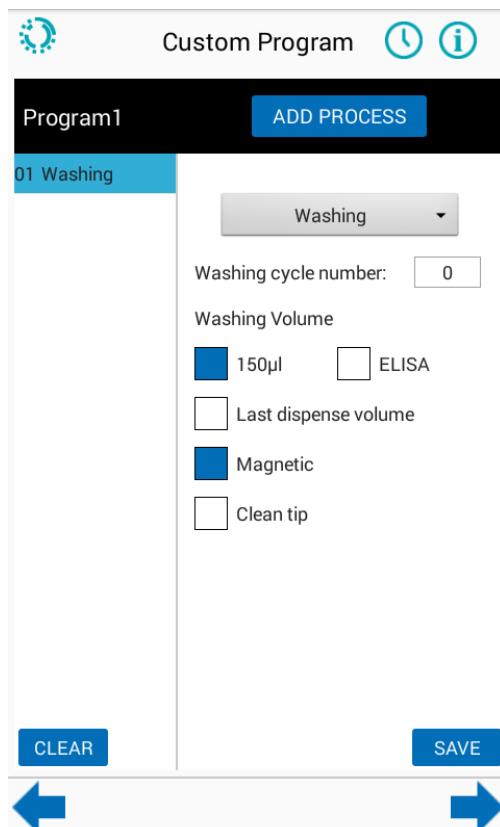


Fig. 16. Parameters of custom washing procedure

SA-PE Reaction: This process includes the fluorescence labeling step with automated SA-PE dispensing. A fixed volume of 50 µL SA-PE will be dispensed into each well, then incubate at 37°C for desired incubation period followed by 4 washing cycles. At the end, additional 150 µL of wash buffer will be dispensed into each well, followed by 10 sec. of shaking to spread πCode evenly. The plate will now be ready for fluorescence analysis using the PlexBio 100 Fluorescent Analyzer.

The incubation period can be adjusted from 1 sec to 23hr 59min 59sec.

Please ensure that enough SA-PE solution is added into V Tray before the program starts. See **Section 5.5 Special Applications** for more detail.

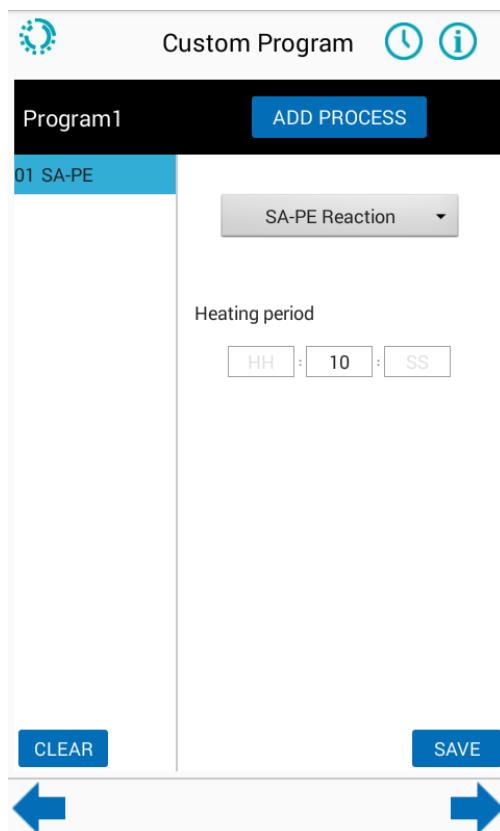


Fig. 17. Parameters of custom SA-PE procedure

Note: The IntelliPlex 1000 πCode Processor dispensing module will take up 50 µL of solution from the V Tray to dispense into selected wells. By default, the V Tray is used for SA-PE. Custom programs may also utilize the V Tray to dispense 50 µL of a solution of choice as long as the reaction is compatible with an incubation at 37°C. For example, immunoassays may dispense Phycoerythrin-labeled detection antibodies instead of SA-PE.

Disperse: This function is to minimize the πCode MicroDiscs aggregations before decoding with the PlexBio 100 Fluorescent Analyzer.

Pause: This function pauses a program to allow opening the front door without triggering the alarm. The ability to open the door without terminating the run provides a chance to check microplate status, add additional reagent manually or refill/ change the solution in the V Tray (we recommend using a new plastic V Tray for each solution).

Once the pause function is reached in an active program, the run will be stopped temporarily. It is only safe to open the door once instructed through a pop-up window in the User Interface. Before continuing the run, ensure that the plate is placed back onto the heating plate, the V Tray is inserted and filled, and the door is closed. The program will continue to the next process after the user clicks the confirm button.

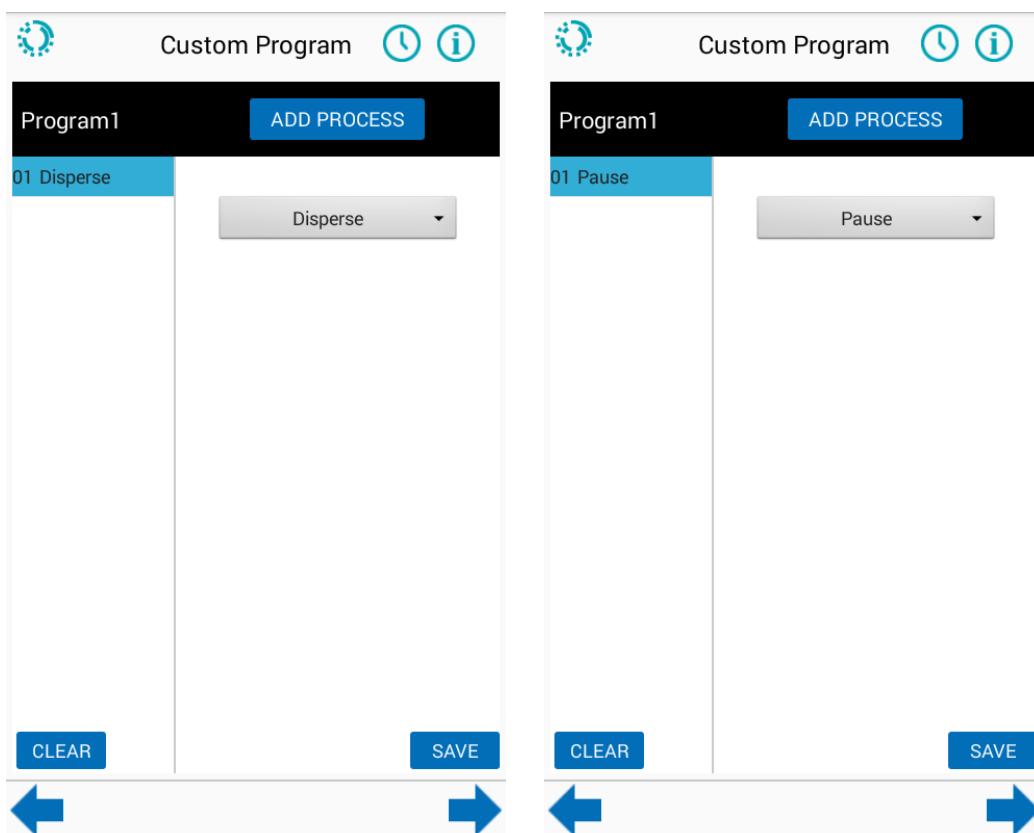


Fig. 18. Disperse and Pause Procedure

5.4.2 How to Create/Edit and Run a Customized New Program

Users can create and save a total of 6 customized programs. Note: Once all six programs have been created, users will need to edit one of the existing programs to create a different custom program.

To setup or edit a program, please follow steps 1 to 9:

- Step 1. Fig. 19: Click **Custom Program** on the home screen and select desired lanes for processing and click **Next**.
- Step 2. Fig. 20: Select one program to create/edit/run from Program 1 to 6 selection.
 - If the program already exists, go to step 9.
 - If the program needs editing, go to step 6.
 - If the program needs to be created, go to step 3.
- Step 3. Fig. 21: Click **ADD PROCESS** and choose the desired procedure from the drop-down menu on the right side.
- Step 4. Fig. 22: Set detailed parameters for each procedure like temperature, time, washing cycles, etc., and save the procedure (optional).
- Step 5. Repeat Step 3 and 4 to add all desired procedures to the custom program.
- Step 6. Click on any of processes on the left to edit its parameters.
- Step 7. Confirm all the procedures and the order are correct.
- Step 8. Click **SAVE** to save the program (optional).
- Step 9. Click ➔ to run the custom assay processes.

NOTE:

1. To delete a process that was added to the program, press on the appropriate process on the touchscreen for several seconds. A pop-up window will appear and confirmation to delete the process from the program is required.
2. Before running a custom program, make sure there is enough water and wash buffer (refer to section 5.5.2) and SA-PE solution (refer to section 5.5.1) in the dedicated containers, and the waste bottle is empty.
3. Once a custom program is started, the real-time operational status of the assay is displayed on the touchscreen until the run is completed (Fig. 23).

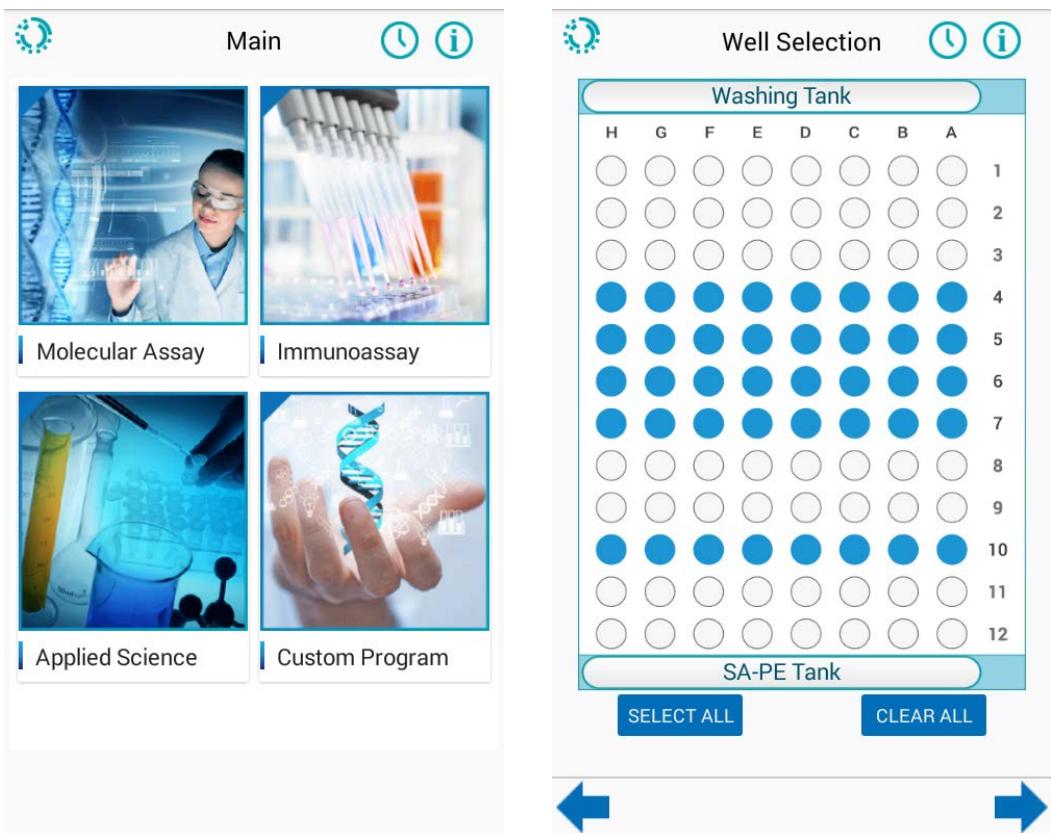


Fig. 19. Custom Program Home Screen and Well Selection

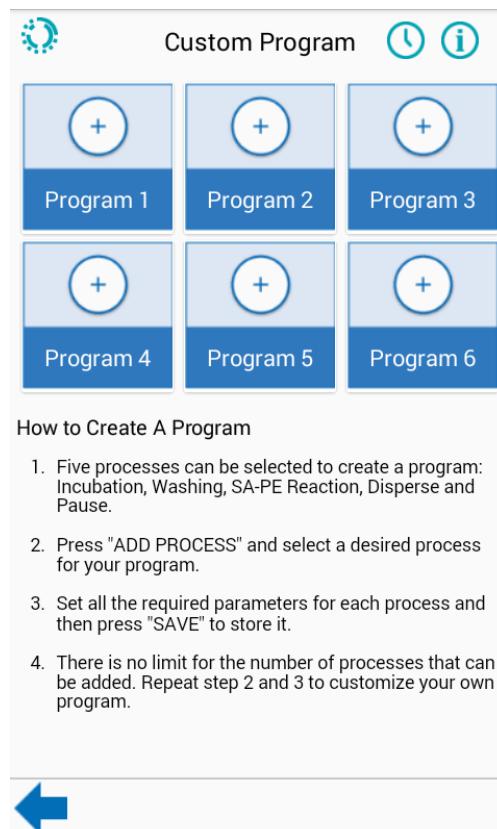


Fig. 20. Custom Program 1 to 6

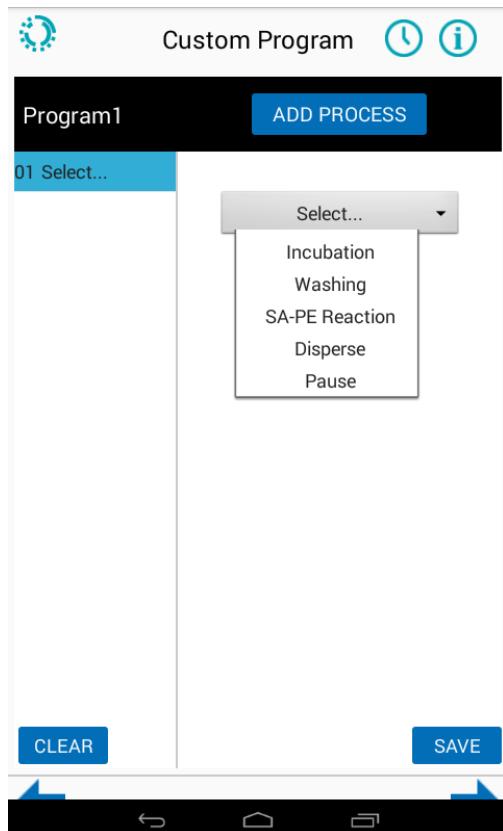


Fig. 21. Select desired procedures to set up a process

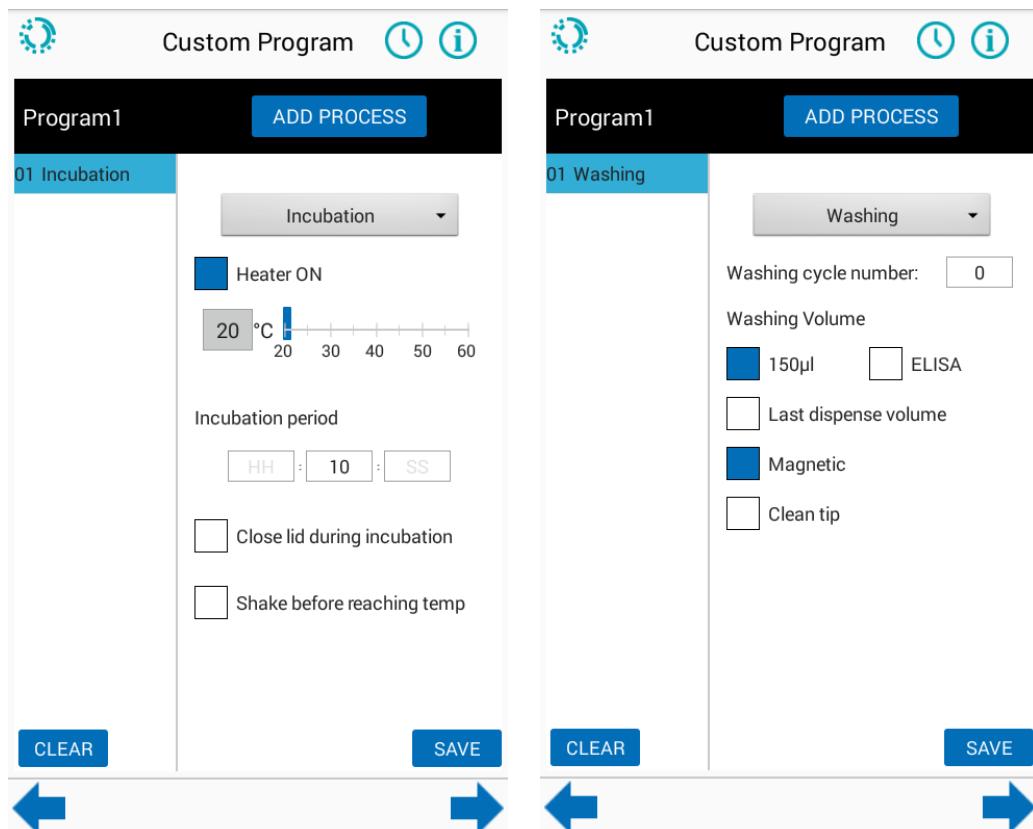


Fig. 22. Set detailed parameters for each process



Fig. 23. Custom Program Process and Real-time Display

5.5 Special Applications

5.5.1 SA-PE Solution

This section describes the procedures for SA-PE solution preparation.

Preparation of SA-PE solution is required for all built-in PlexBio assays or any customized assays which utilizes πCode MicroDisc technology. Before starting any run, the appropriate amount of SA-PE solution must be added to the V Tray in the IntelliPlex 1000 πCode Processor.

The position of SA-PE tank is shown in Fig. 24. For each run, place a new clean plastic V Tray in the SA-PE tank and add enough SA-PE solution. The required volume of SA-PE solution per selected lane (8 wells, A to H; see schematic representation below) in an assay is **400 μL**. Please note that the dead volume of the V Tray is **500 μL** for up to 6 selected lanes or **800 μL** for more than 6 selected lanes (7-12 lanes). The minimum quantity of SA-PE solution required is **one lane = 400 μL + 500 μL = 900 μL**. Please refer to the overview table below for required SA-PE volumes based on numbers of lanes selected.

Calculation Example:

For a 3-lane reaction, the SA-PE solution needed is

$$400 \mu\text{L} \times 3 \text{ lanes} + 500 \mu\text{L} = 1.7 \text{ mL}$$

Required SA-PE Solution by Lane(s):

Number of Processed Lane(s)	Required SA-PE Solution (μL)
1	900
2	1300
3	1700
4	2100
5	2500
6	2900

Number of Processed Lane(s)	Required SA-PE Solution (μL)
7	3600
8	4000
9	4400
10	4800
11	5200
12	5600

Note: Do not reuse the leftover SA-PE solution. Replace a new V Tray with every assay run.

A-H is defined as a lane.

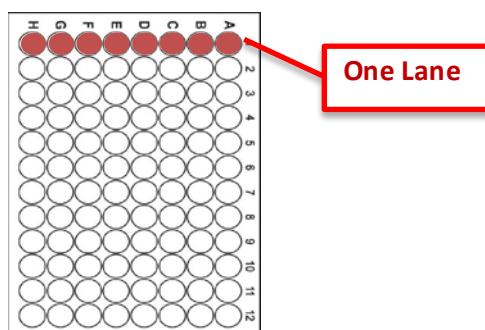




Fig. 24. Positions of the Washing and SA-PE Tanks

5.5.2 Wash Buffer

This section describes the procedures for Wash buffer preparation.

Preparation of Wash buffer is required for all built-in PlexBio assays. Before starting a run, sufficient amount of Wash buffer must be added to the Wash Buffer bottle connected to the IntelliPlex 1000 πCode Processor.

Wash buffer purchased from PlexBio is 10X concentrated. Preparation of 1X Wash Buffer (10-fold dilution of 10X Wash buffer with ddH₂O) is required before usage with the IntelliPlex 1000 πCode Processor. The prepared 1X Wash Buffer can be used for up to 1 week.

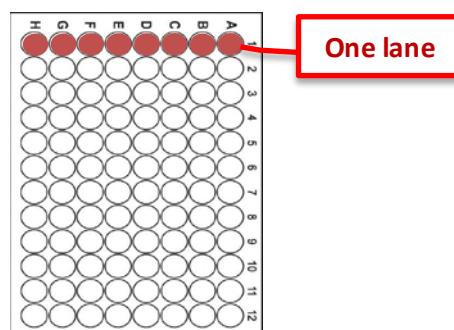
Do not use the buffer if visible contamination (e.g. fungus) occurs. Please discard of contaminated Wash Buffer according to local requirements and wash the fluid path and buffer bottles several times with 70% ethanol (refer to section 7.2.1).

In a custom program, the amount of wash buffer required per row depends on the selection of program parameters (such as number of wash cycle and selection of “clean tip” option). We recommend a fill level of at least 500 mL Wash Buffer for all assays with 7 or less wash cycles and a fill level of 1000 mL Wash Buffer for programs utilizing more than 7 wash cycles.

For PlexBio’s DNA/RNA program, the Wash Buffer consumption is as following:

Procedure	Wash Buffer Consumption
Self-test	50 mL
DNA/RNA program for 1 lane, up to 8 tests (3 wash cycles and 1 SAPE reaction)	150 mL
DNA/RNA program for 3 lanes, up to 24 tests (3 wash cycles and 1 SAPE reaction)	220 mL
DNA/RNA program for 6 lanes, up to 48 tests (3 wash cycles and 1 SAPE reaction)	340 mL
DNA/RNA program for 12 lanes, up to 96 tests (3 wash cycles and 1 SAPE reaction)	540mL

A-H is defined as a lane.



Chapter 6. System Settings

To get to the **System Menu**, click on  (top left screen corner).

The Settings menu is shown in Fig. 25. Each section is described in detail below.

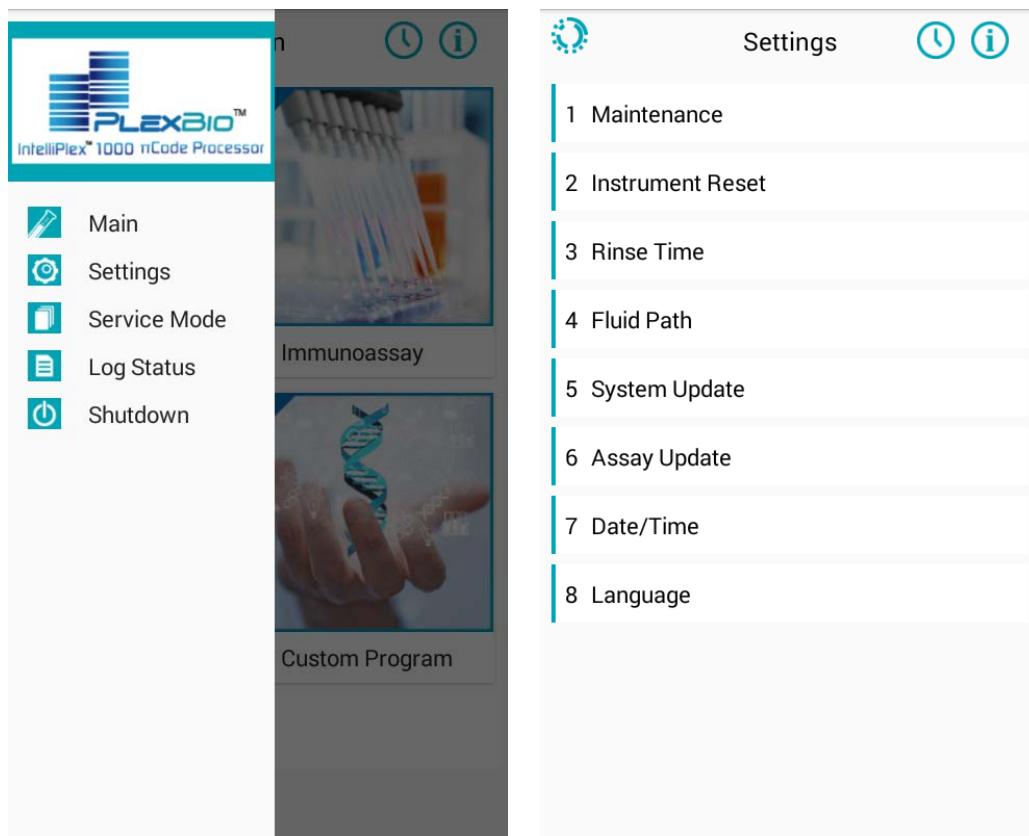


Fig. 25. The settings menu

6.1 Maintenance

This section describes how to renew the solution U Trays and clean the manifold (Fig. 26).

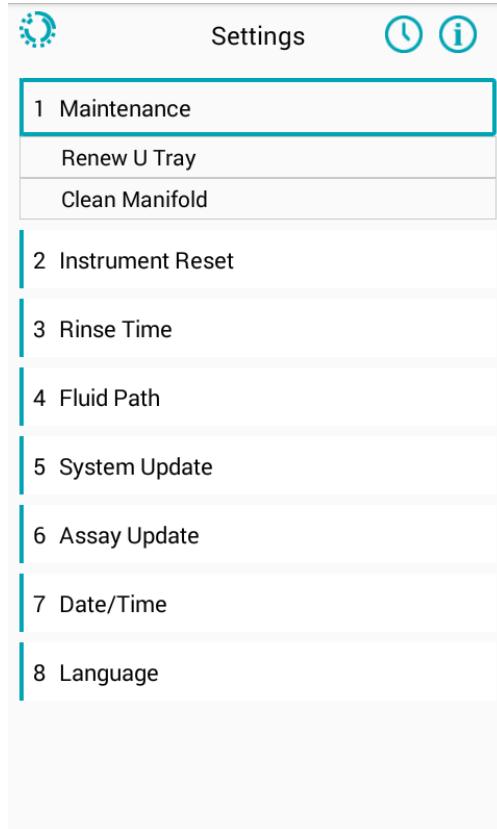


Fig. 26. Maintenance from tool menu

6.1.1 Renew U Tray

There are two tanks - the **washing tank (U Tray)** and the **SA-PE tank (V Tray)** (Fig. 27). Only the transparent U and V Tray is replaceable, do not tamper with the fixed white tank modules from the instrument. We recommend replacing the **U Tray** once a month to keep the tray clean and reduce the possibility of salts crystal formation or contamination. While the **V Tray** can be replaced directly (please dispose/replace after every run), replacing the **U Tray** requires the maintenance function in the settings menu.

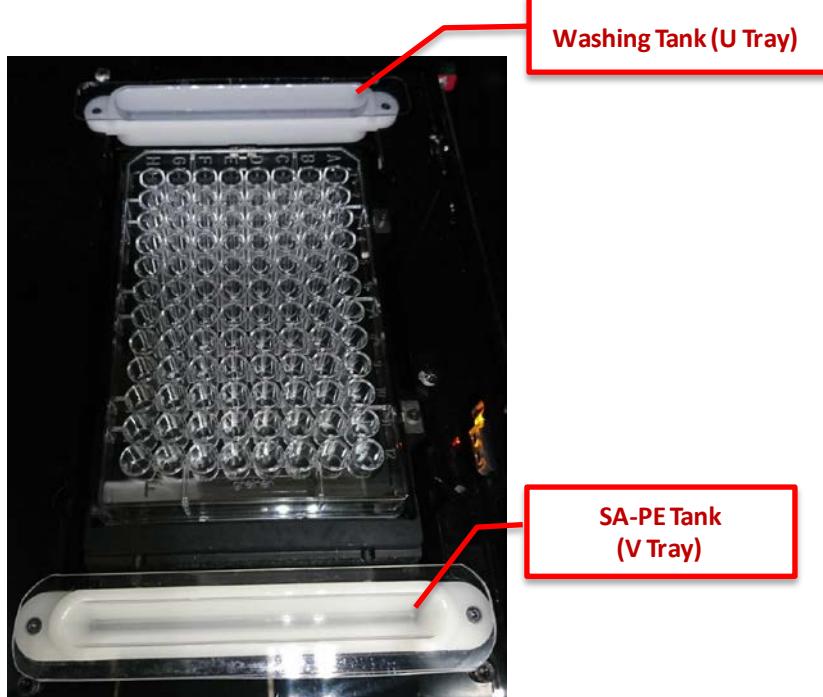


Fig. 27. Positions of the Washing and SA-PE Tanks

To renew the **U Tray**, please follow the steps below:

- Step 1. Open the **System Menu** 
- Step 2. Select **Setting** on the menu page.
- Step 3. Click on the **Maintenance** button.
- Step 4. Choose **Renew U Tray** (Fig. 28) and the manifold will move backwards to reveal the washing tank for replacing the U Tray.
- Step 5. Please remove the plastic U Tray carefully and avoid spilling of any liquid that might still be at the bottom of the tray.
- Step 6. Place a new tray into the white manifold. Ensure proper placement.

Note: It is recommended to renew U Tray once a month to reduce the possibility of contamination or crystal formation.

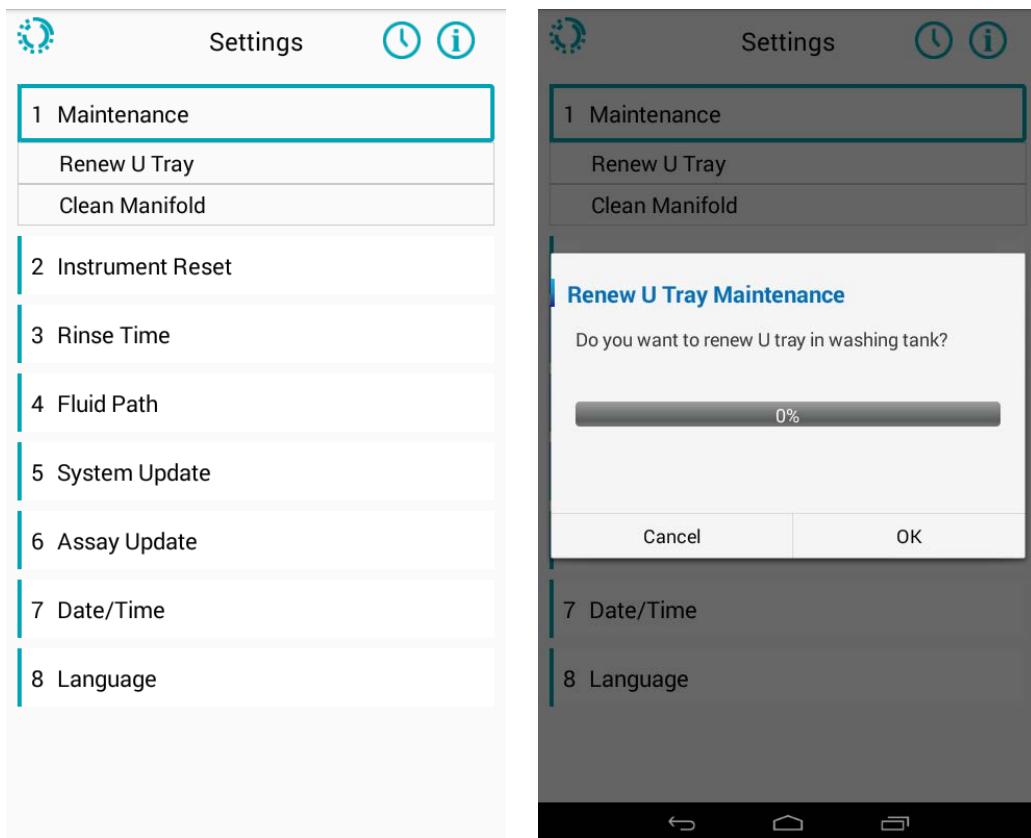


Fig. 28. Renew U Tray

Note: Never operate the IntelliPlex 1000 πCode Processor without the transparent U Tray placed in the white tank module. Operating the IntelliPlex 1000 πCode Processor without the plastic U Tray placed in the white module may damage the IntelliPlex 1000 πCode Processor. We recommend to visually confirm the presence of the plastic U Tray before operating the IntelliPlex 1000 πCode Processor.

6.1.2 Clean Manifold

Manifold cleaning helps to keep the manifold in good working order. If crystals do form and block the manifold tips, or visible dust develops on the manifold tips, follow the steps described below (Fig. 29), use the needles provided in the accessory box to clean the manifold tips.

- Step 1. Remove V Tray from SA-PE tank.
- Step 2. Open the **System Menu** 
- Step 3. Select **Setting** on the menu page.
- Step 4. Click on the **Maintenance** button.
- Step 5. Select **Clean Manifold** and then click **OK** in the pop-up window. The dispensing module will move forward to allow access to the manifold.
- Step 6. After manifold cleaning, perform **Instrument Reset** (see below).

Note: Manifold tips disassembly is restricted to qualified engineer only. If there is any precipitation or dust on manifold tips which cannot be cleaned away, contact **PlexBio Co., Ltd.** or local distributors for services.

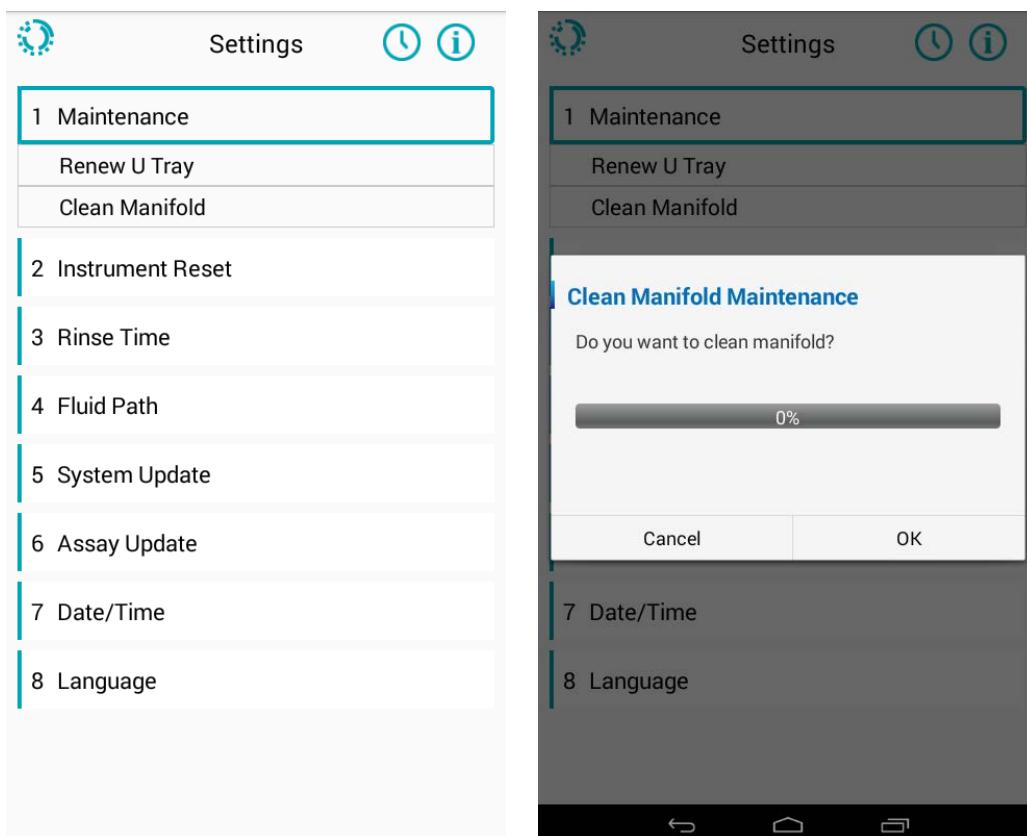


Fig. 29. Clean the manifold

6.2 Instrument Reset

This function returns the manifold, the thermal lid, and the magnetic plate back to their original positions.

- Step 1. Select **Setting** in the menu.
- Step 2. Choose **Instrument Reset** to reset all modules.
- Step 3. Click **OK** in the pop-up window.

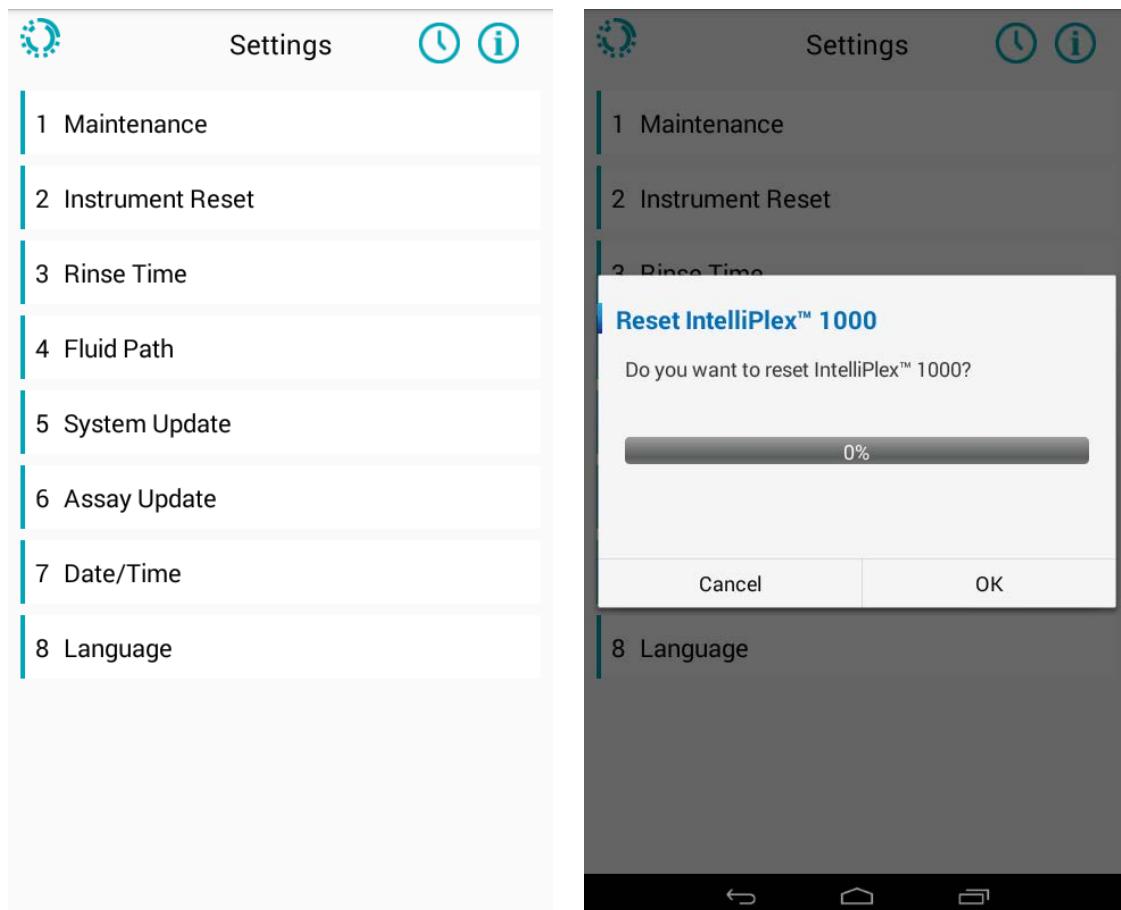


Fig. 30. IntelliPlex1000 instrument reset

6.3 Rinse Time

The **RINSE** function is used to keep pumps and tubes wet and to prevent salt crystal formation which could lead to clogging of the fluid path and/or damage the pump.

By default, the **RINSE** function will execute after the IntelliPlex 1000 πCode Processor was idle for 2 hours, and as long as the instrument remains idle, the system will perform **RINSE** function repeatedly. The **RINSE** function will rinse the tubes with ddH₂O. Once the **RINSE** function is completed, a pop-up window will appear on the screen instructing to start the **PRIME** function, which will prime the tubing with Wash Buffer. The **PRIME** function can be performed immediately after the **RINSE** function or later but must be completed before performing the next run. Please make sure the wash buffer and waste bottle fill levels are adequate before running **PRIME**.

To adjust the idle time triggering the **RINSE** function follow the steps below:

- Step 1. Open the **System Menu** 
- Step 2. Select **Setting** on the menu page.
- Step 3. Select **Rinse Time** in the setting menu.
- Step 4. Set up the **Rinse Time**. The automated rinse can be set between 10 minutes or 24 hours.

Note: We recommend keeping the default settings. Please keep in mind that the instrument will perform another **RINSE** cycle if the instrument remains idle for 2 hours after the **PRIME** function was executed.

6.4 Fluid Path

There are three options: **RINSE** (ddH₂O), **PRIME** (wash buffer) or **RINSE/PRIME**. **RINSE** dispenses liquid from the ddH₂O inlet, while **PRIME** dispenses liquid from the wash buffer inlet. These functions are used during monthly maintenance and long-term shutdown procedure (see Chapter 7 for details)

- Step 1. Open the **System Menu** 
- Step 2. Select **Setting** on the menu page.
- Step 3. Select **Fluid Path** on the tool menu.
- Step 4. Choose **RINSE, PRIME** or **RINSE/PRIME**.
- Step 5. Press **OK** to start running.

Note: Before performing any of the function, please ensure that the ddH₂O and Wash Buffer bottles are adequately filled, and the Waste bottle is empty.

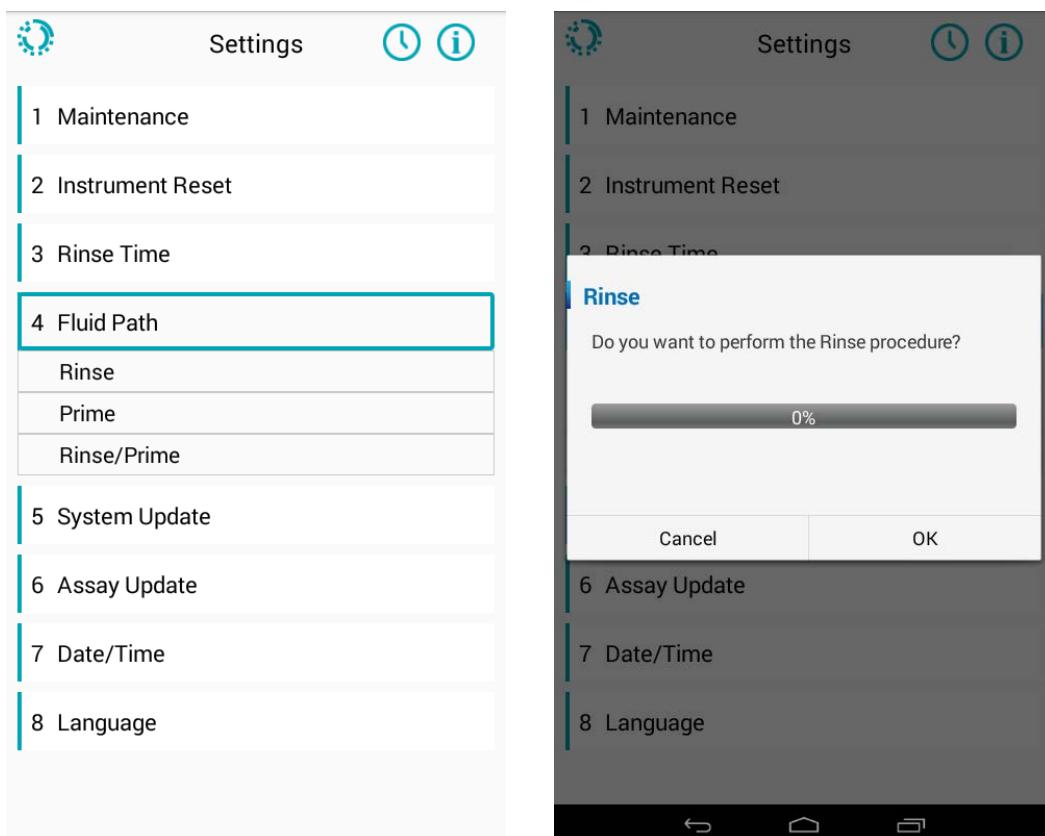


Fig. 31. Fluid path process

6.5 System Update

This function is restricted to qualified engineer only. The software version will be checked during maintenance by engineer. Contact PlexBio Co., Ltd. or local distributors for more information.

6.6 Assay Update

This function allows to update the built-in assay protocols.

PlexBio Co., Ltd or your local distributor will inform you once upgrades become available and provide further instruction. Updates are uploaded using the USB port below the touch screen of the IntelliPlex 1000 πCode Processor.

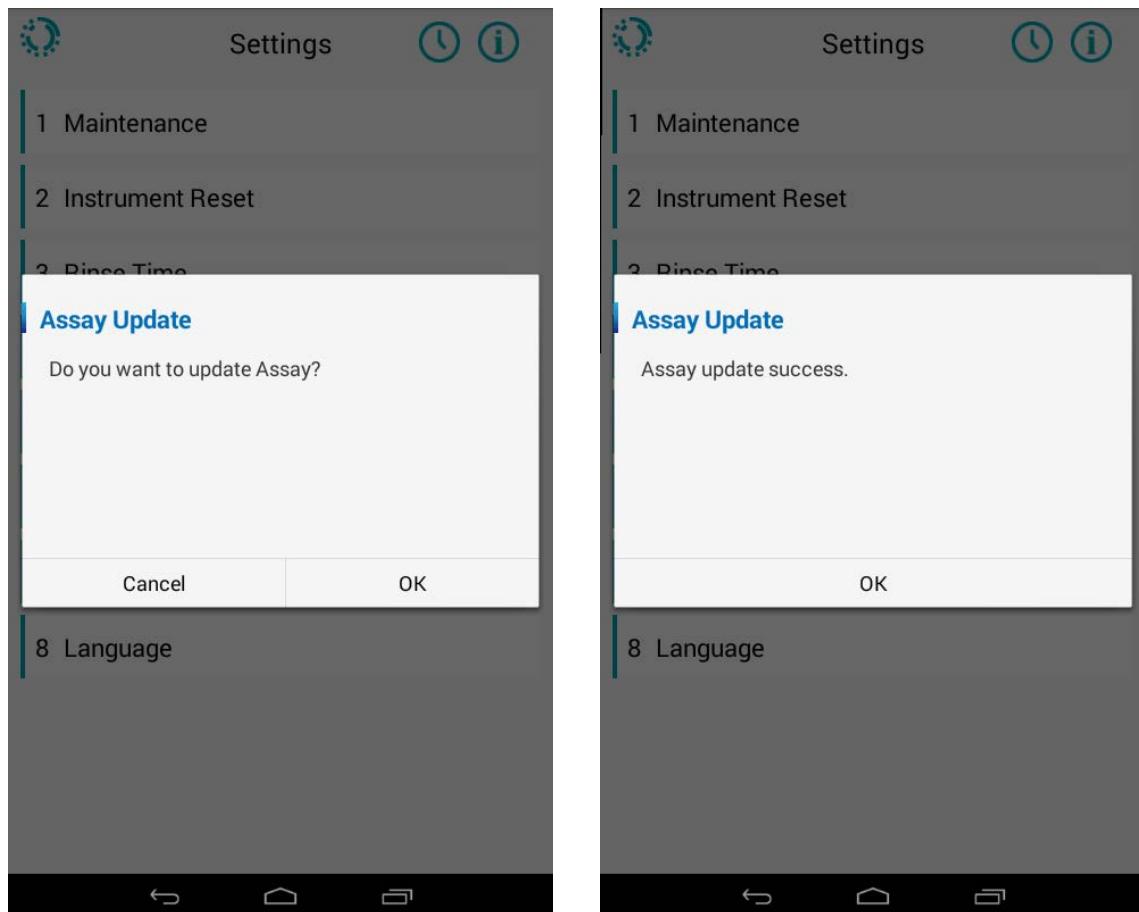


Fig. 32. Update Assay Protocols

6.7 Date/Time

Setup the current Date and Time for the instruments.

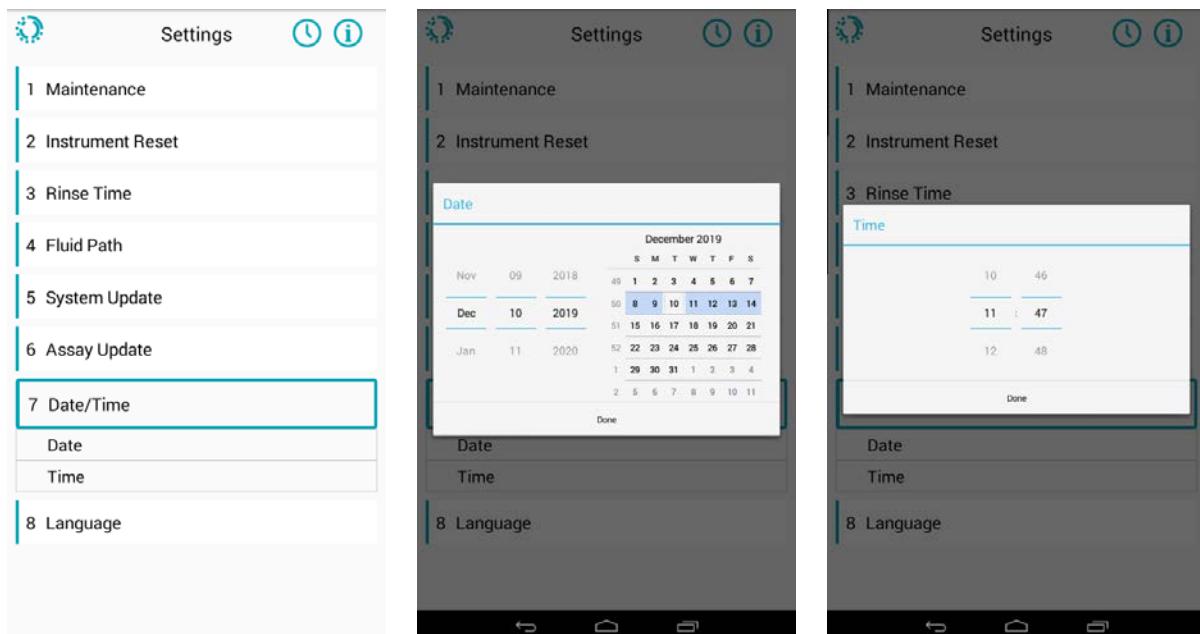


Fig. 33. Date and time setting

6.8 Language

Set the language to English or Simplified Chinese.

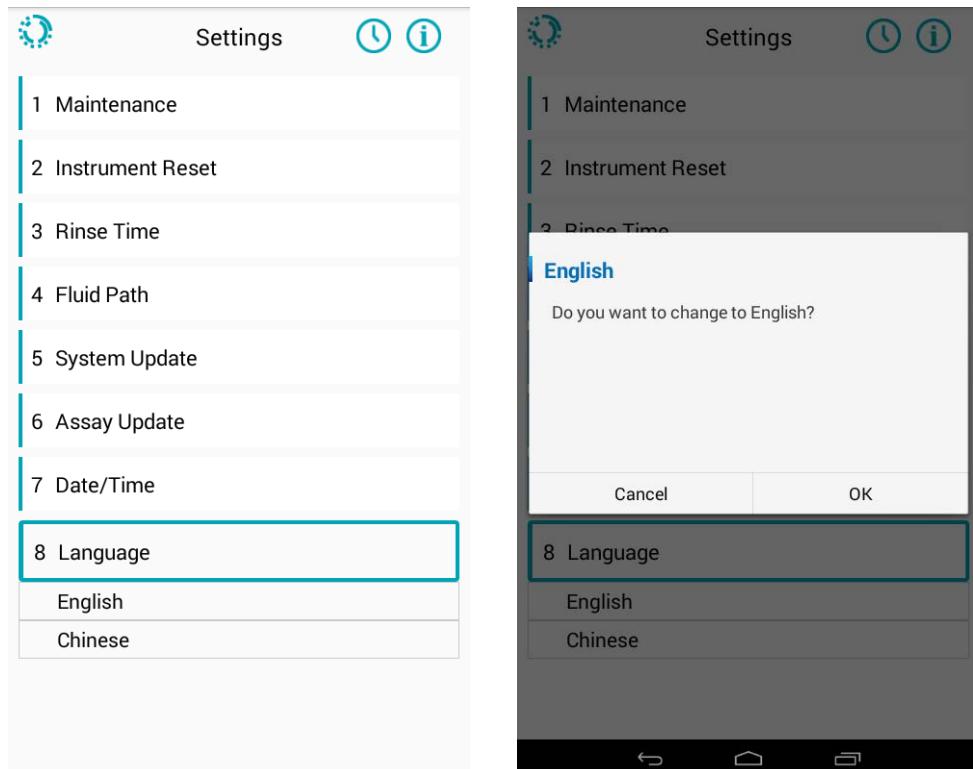


Fig. 34. Language setting

6.9 Service Mode

The Service Mode is intended for use by a qualified service engineer. Do not access the service mode unless specific instructions are given by PlexBio technical support.

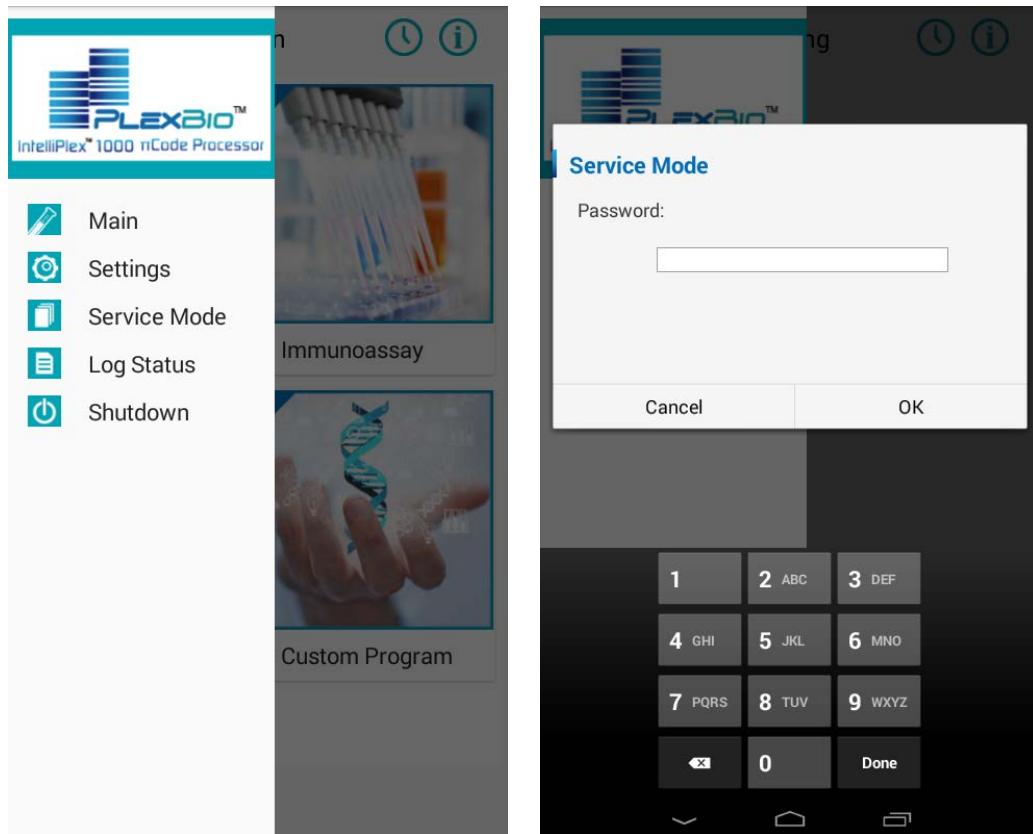


Fig. 35. Service Mode

6.10 Log Status

To view the system log history:

- Step 1. Select the **Log Status** on the home screen menu.
- Step 2. Choose the date and time to check the detailed log.
- Step 3. Click **DISPLAY** to check the detailed log.

To export log files:

Step 1. Prepare an USB drive with FAT32 format and at least 2GB capacity available.

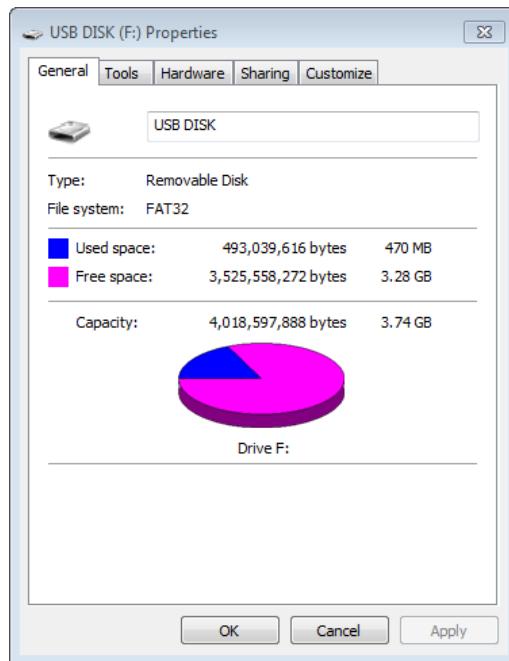


Fig. 36. USB drive requirements

Step 2. Create a new folder named “**PlexbioUpdate**”.

Step 3. Insert the USB drive into the USB port below the touch screen.

Step 4. Pull out the menu and select **Log Status**.

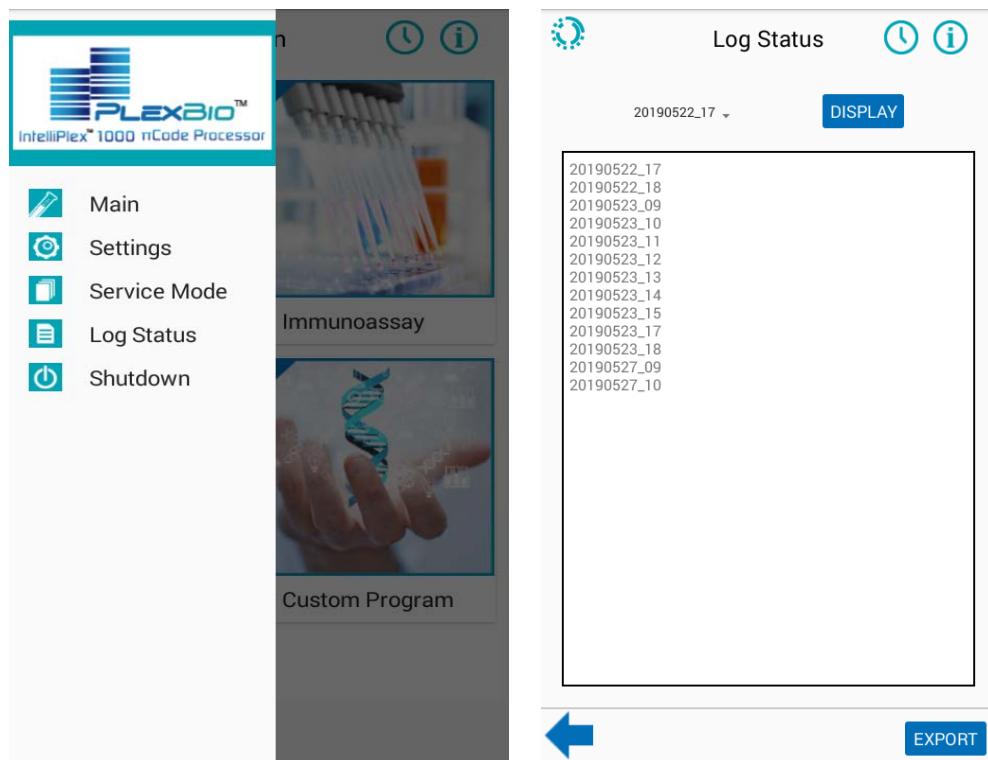


Fig. 37. Log status menu

Step 5. Click **EXPORT** on the bottom right to export log files.

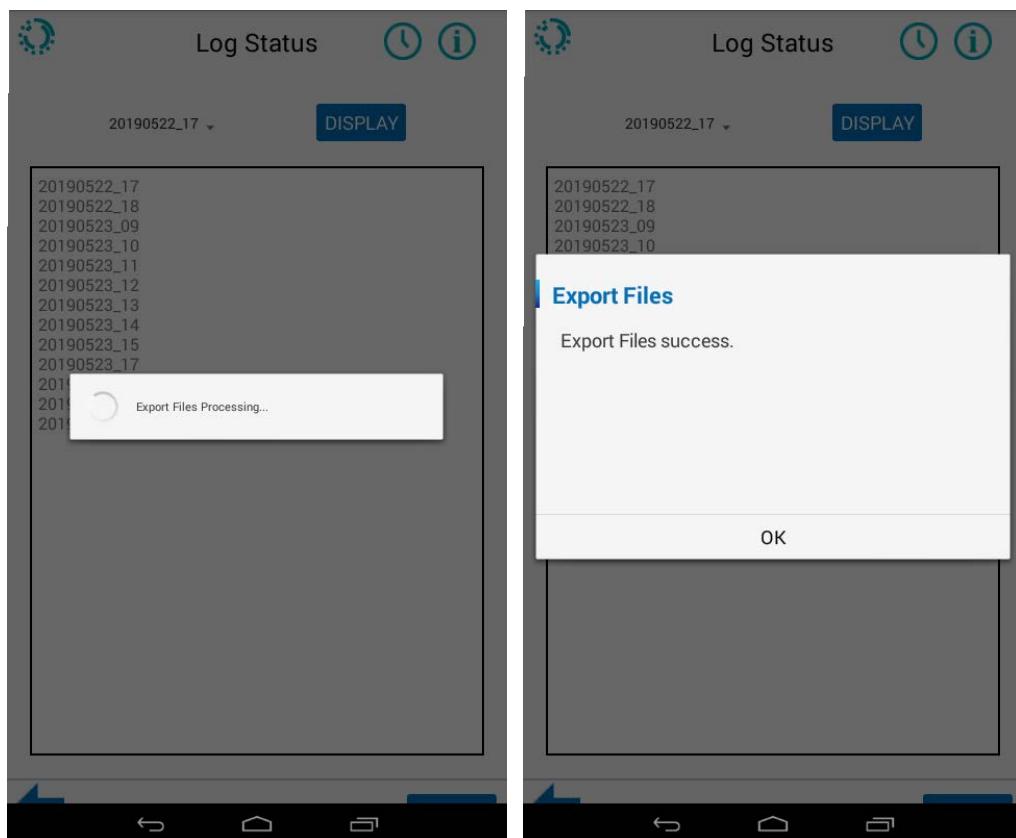


Fig. 38. Export log files

Step 6. Pull out the USB drive after the process is completed.

Step 7. Connect the USB drive to a computer to check the files saved in “Logs” file folder.

6.11 Shutdown

After finishing using the IntelliPlex 1000 Processor, it is important to shut down the system. To do so, run the **Shutdown** program. The **Shutdown** procedure will ensure that the pump and tubes are rinsed, and all modules are at their proper position. **Never** turn off the power on the backside of the instrument without running the **Shutdown** program first.

- Step 1. Open the **System Menu** 
- Step 2. Select **Shutdown** on the menu.
- Step 3. Press **OK** to run the auto-rinse followed by the system turning off.
- Step 4. After the system is off, switch the power button off.

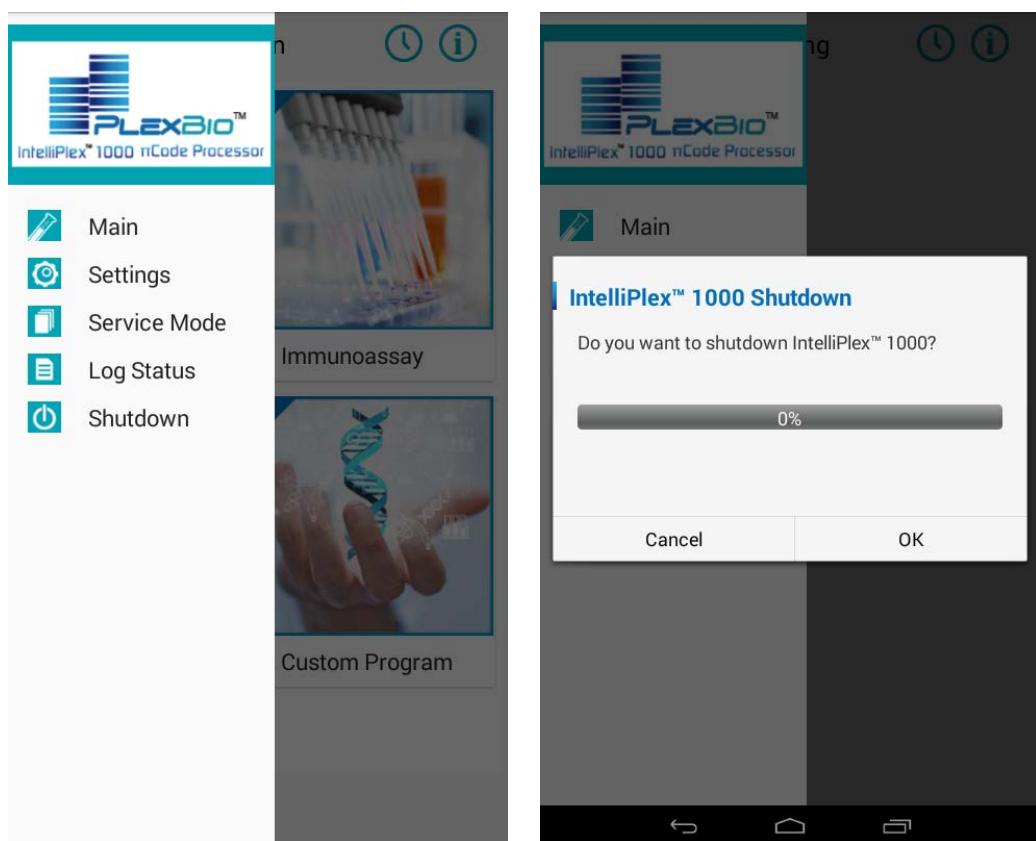


Fig. 39. System shutdown program

Chapter 7. Maintenance

Personnel that use, maintain, or clean IntelliPlex 1000 πCode Processor should be trained in standard laboratory safety practices and should follow those practices when handling the instrument.



Biohazard

Samples and waste fluid may contain biohazardous material. Where exposure to biohazardous material exists, follow appropriate bio-safety procedures, use personal protective equipment, and use ventilation devices.



General Cautions

Users should not perform any maintenance or cleaning of the electrical components in the system without training.

7.1 Recommended Maintenance Schedule

Regular care and maintenance of IntelliPlex 1000 πCode Processor will ensure optimal system performance. It is required to follow the schedule below. Operating the IntelliPlex 1000 πCode Processor without proper maintenance will void its warranty.

Please read and follow the section **7.2 As Needed Maintenance** below carefully. For more information on Preventive Maintenance or Extended Warranties, please contact Customer Service of PlexBio Co., Ltd. or qualified local distributors.

Action	User		Qualified Specialist
	Daily	Monthly	Yearly
Check/Empty waste bottles	✓		
Long-term Shutdown		Varies	
Replace U Tray from Washing Tank		✓	
Clean bottles		✓	
Clean external surface		✓	
Clean fluid path tubes with 70% ethanol		✓	
Lubricate the stage guideway to prevent stage positioning error or motor failure.			✓
Replace the plastic tips			✓
Re-position all axes of moving modules			✓
Calibrate waterway performance			✓
Calibrate heater performance			✓

Important: Depending on users' assays, some or all the maintenance procedures are more

frequently required than recommended in the schedule.

7.2 As Needed Maintenance

7.2.1 Clean Fluid Path Tubes with 70% Ethanol

It is required to clean the fluid path at least once a month:

- Step 1. Disconnect the ddH₂O and wash buffer bottle lids from their respective bottles.
- Step 2. Empty bottle by discarding all solutions. Rinse Wash Buffer bottle several times with water.
- Step 3. Use a bottle containing 800mL 70% ethanol (if not dedicated ethanol bottle is available, the ddH₂O bottle can be filled with 800mL 70% ethanol and used instead) and connect the ddH₂O bottle lid. Ensure the straw is submerged in ethanol.
- Step 4. Confirm that the Waste Bottle is connected and has sufficient space.
- Step 5. Run the **Rinse** cycle of the Fluid Path on the setting menu **four times** (refer to section 6.4).
- Step 6. Remove the ddH₂O bottle lid and place on clean paper towel. Connect the wash buffer lid to the bottle with 70% ethanol. Ensure the straw is submerged in ethanol.
- Step 7. Run the **Prime** cycle of the Fluid Path on the setting menu **four times** (refer to section 6.4).
- Step 8. Remove the wash buffer lid from the bottle and place on clean paper towel.
- Step 9. Run the **Rinse/Prime** cycle of the Fluid Path on the setting menu **four times**. This step will pump air through the tubing to displace any remaining liquid.
- Step 10. Disconnect the Waste Bottle Lid and empty the Waste Bottle, rinse several times with water.
- Step 11. Clean all lids with 70% ethanol and let air dry.
- Step 12. Clean all bottles with 70% ethanol and let air dry.
- Step 13. Assemble all bottle and lids with the IntelliPlex 1000 πCode Processor.
- Step 14. Fill the ddH₂O and Wash Buffer bottles with at least 500mL ddH₂O or Wash Buffer.
- Step 15. Perform **Rinse/Primer** two times (refer to section 6.4).
- Step 16. The IntelliPlex 1000 πCode Processor is operational.

Note: The “Clean Fluid Path Tubes” operation must be performed using 70% ethanol. The use of BLEACH and other chemicals is not permitted.

7.2.2 Clean and Disinfect Surfaces

We recommend cleaning the exterior surfaces of the IntelliPlex 1000 πCode Processor as needed. The surfaces can be cleaned with water, 70% ethanol or a mild detergent (followed by water wipe to remove any residual detergent).

To disinfect contaminated areas, remove all contaminant first. Disinfect the area using a 0.1% bleach solution for 5 minutes, then rinse well with water. Please note that interior spills must be handled with extreme care. Do not use excessive volumes, liquid must not gain access to the interior of the instrument. Caution: the heating plate and lid may be hot.

7.2.3 Renew U Tray

It is required to renew the U Tray at least once per month. Please refer to section 6.1.1.

7.2.4 Long-term Shutdown

We recommend performing **Long-term Shutdown** if the IntelliPlex 1000 πCode Processor will not be in operation for more than a week or if it cannot be switched on/off at least once a week.

The **Long-term Shutdown** procedure cleans the fluid path with water to remove all Wash Buffer followed by a 70% ethanol rinse to reduce the possibility of salt crystal formation and bacterial/fungal growth in the fluid path.

- Step 1. Remove the plastic V Tray.
- Step 2. Disconnect the ddH₂O and wash buffer bottle lids from their respective bottles
- Step 3. Empty bottle by discarding all solutions. Rinse Wash Buffer bottle several times with water.
- Step 4. Use a bottle containing 800mL 70% ethanol (if not dedicated alcohol bottle is available, the ddH₂O bottle can be filled with 800mL 70% ethanol and used instead) and connect the ddH₂O bottle lid. Ensure the straw is submerged in ethanol.
- Step 5. Confirm that the Waste Bottle is connected and has enough space.
- Step 6. Run the **Rinse** cycle of the Fluid Path on the setting menu **four times** (refer to section 6.4).
- Step 7. Remove the ddH₂O bottle lid and place on clean paper towel. Connect the wash buffer lid to the bottle with 70% ethanol. Ensure the straw is submerged in ethanol.
- Step 8. Run the **Prime** cycle of the Fluid Path on the setting menu **four times** (refer to section 6.4).

- Step 9. Remove the wash buffer lid from the bottle and place on clean paper towel.
- Step 10. Run the **Rinse/Prime** cycle of the Fluid Path on the setting menu **four times**. This step will pump air through the tubing to displace any remaining liquid.
- Step 11. Remove the plastic U Tray (refer to section 6.1.1).
- Step 12. Run the **Shutdown** program to turn off the instrument.
- Step 13. Switch the power off.
- Step 14. Disconnect the power cord from the power plug.
- Step 15. Disconnect the Waste Bottle Lid and empty the Waste Bottle, rinse several times with water.
- Step 16. Clean all lids with 70% ethanol and let air dry.
- Step 17. Clean all bottles with 70% ethanol and let air dry.
- Step 18. Assemble all bottle and lids with the IntelliPlex 1000 πCode Processor. Close the air valves of the ddH₂O and Wash Buffer lids.

7.2.5 Restarting after Long-term Shutdown

To initiate the IntelliPlex 1000 πCode Processor after Long-term Shutdown:

- Step 1. Open the air valves of the ddH₂O and Wash Buffer lids.
- Step 2. Do **not** fill the ddH₂O and Wash Buffer bottles.
- Step 3. Ensure that the on/off switch in the rear panel of the instrument is in the off position.
- Step 4. Plug the power cord into the back of the instrument and switch on the IntelliPlex 1000 πCode Processor.
- Step 5. Perform self-test (the system will pump air during self-test).
- Step 6. Place a new plastic U Tray (refer to section 6.1.1).
- Step 7. Fill the ddH₂O and Wash Buffer bottles with at least 500mL ddH₂O or Wash Buffer.
- Step 8. Perform **Rinse/Prime** two times (refer to section 6.4).
Visually confirm the uptake of ddH₂O or Wash Buffer and confirm discharge into waste bottle. If no ddH₂O or Wash Buffer uptake is observed, repeat **Rinse/Prime** up to four additional times. If still no uptake is observed, please contact Customer Service of PlexBio Co., Ltd. or qualified local distributors. If no ddH₂O or Wash Buffer uptake is observed but no discharge into waste bottle, perform system shutdown, switch the instrument and contact Customer Service of PlexBio Co., Ltd. or qualified local distributors.
- Step 9. The IntelliPlex 1000 πCode Processor is operational.

7.2.6 Connecting the Components

To connect the components:

- Step 1. Take out all the accessories including bottles, tubes and the AC power cord.
- Step 2. Plug the power cord into the back of the instrument.
- Step 3. Ensure that the on/off switch in the rear panel of the instrument is in the off position.
- Step 4. Fill up the wash buffer bottle with wash buffer and fill up the ddH₂O bottle with distilled water.
- Step 5. Connect the buffertubes (Wash buffer and Distilled water) to the inlet connectors on the rear panel of the instrument and to the corresponding bottle as well. See the Fig. 4 for the connected diagrams.

7.2.7 Fuse Replacement

If the fuse in the instrument is broken, please follow these steps to replace it:

- Step 1. Pull out the fuse holder from the power socket.
- Step 2. Take out the broken fuse and replace it with a new one from the accessory box.
- Step 3. Push the fuse holder back to its original place.



Fig. 40. Fuse holder



Fig. 41. Fuse and the fuse holder

Chapter 8. Troubleshooting

Item	Error Code	Description	Solution
1	1001	The temperature of bottom heater is higher than 65°C. The system will be locked.	Contact PLEXBIO or local distributors for service.
2.	1002	The temperature of top heater is higher than 65°C. The system will be locked.	Contact PLEXBIO or local distributors for service.
3.	1003	Abnormality is detected from the bottom heater sensor. The system will be locked.	Contact PLEXBIO or local distributors for service.
4.	1004	Abnormality is detected from the top heater sensor. The system will be locked.	Contact PLEXBIO or local distributors for service.
5	1005	The temperature cannot reach $\pm 1.5^{\circ}\text{C}$ of configured temperature in 3 minutes during an incubation process. The system will be locked.	Contact PLEXBIO or local distributors for service.
6	1103	The temperature of bottom heater is detected to be higher than 95 °C. The system will be locked.	Contact PLEXBIO or local distributors for service.
7	1104	The temperature of top heater is detected to be higher than 95°C. The system will be locked.	Contact PLEXBIO or local distributors for service.
8	2001	The shaker motor does not return to the defined home position.	Reboot the machine and perform self-test. Contact PLEXBIO or local distributors for service if the error continues.
9	2005	The rpm of BLDC (shaking motor) is instable.	Reboot the machine and perform self-test. Contact PLEXBIO or local distributors for service if the error continues.
10	3001	Y axis moves abnormally. The system will be locked.	Contact PLEXBIO or local distributors for service.
11	3101	Z axis moves abnormally. The system will be locked.	Contact PLEXBIO or local distributors for service.
12.	3201	Z _P axis moves abnormally. The system will be locked.	Contact PLEXBIO or local distributors for service.
13.	3301	Z _M axis moves abnormally. The system will be locked.	Contact PLEXBIO or local distributors for service.
14	3401	The heat lid moves abnormally. The system will lock from further use.	Contact PLEXBIO or local distributors for service.
15	6001	The temperature of bottom heater is detected to be 1.5°C higher than the configured temperature during the period of 3 minutes after incubation process started to the end of incubation. The system will be locked.	Contact PLEXBIO or local distributors for service.

Item	Error Code	Description	Solution
16	6002	The temperature of bottom heater is detected to be 1.5°C lower than the configured temperature during the period of 3 minutes after incubation process started to the end of incubation. The system will be locked.	Contact PLEXBIO or local distributors for service.
17	6101	The temperature of top heater is detected to be 1.5°C higher than the configured temperature during the period of 3 minutes after incubation process started to the end of incubation. The system will be locked.	Contact PLEXBIO or local distributors for service.
18	6102	The temperature of top heater is detected to be 1.5°C lower than the configured temperature during the period of 3 minutes after incubation process started to the end of incubation. The system will be locked.	Contact PLEXBIO or local distributors for service.
19	6501	The temperature appears to be lower than operation requirement (<18°C) during the temperature check when the instrument is turned on.	Wait until the temperature rises to requirement if the environmental temperature is within suitable range.
20	6502	The temperature does not reach the operation requirement within an hour after error 6501 occurs. The instrument should be operated under 18-32°C.	Shutdown the instrument. Make sure the environmental temperature rises to suitable range and reboot the instrument.
21	6503	The current temperature is too high. The configured temperature has to be at least 5 °C greater than current temperature to successfully start the program.	Wait until the heater cools down naturally, or check the environmental temperature and try again.
22	5000	Two or more error codes occurs at the same time.	Contact PLEXBIO or local distributors for service.

Chapter 9. Contact Information

PlexBio Co., Ltd.

6F-1, No. 351, Yangguang St., Neihu Dist.,
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Customer Service and Sales

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Order info: order@plexbio.com

Customer Service: service@plexbio.com

Chapter 10. Standard Terms and Conditions for the Use of Products

Contract

The terms and conditions herein (hereinafter referred to as the “Standard Terms”) apply to all of the products and services provided or will be provided by **PlexBio Co., Ltd.** (hereinafter referred to as “**PlexBio**”). Any changes to the Standard Terms shall have no binding effect on **PlexBio** unless the same has been agreed upon by an authorized representative of **PlexBio** in writing. The “Dealer” referred to herein represents **PlexBio** (if the products are purchased from **PlexBio** directly), or the distributor licensed by **PlexBio**. **PlexBio** expressly disagrees with any terms or conditions made by the buyer in the purchase order or any of such which is different from or not included in this Standard Terms.

The buyer's acceptance, unpacking, or use of the products or services shall constitute his/her acknowledgment and acceptance of the Standard Terms unconditionally. Once the buyer opens the packaging of PlexBio products or uses the products or services in any manner, the buyer acknowledges and accepts the Standard Terms unconditionally, and also agrees that the Standard Terms constitute the contract which has binding effect on the buyer under law. Where the buyer disagrees with the Standard Terms, he/she shall contact PlexBio immediately to return the products before using the products or services in any manner, and he/she shall not use the products or services in any manner. **PlexBio** has the right to amend the terms and conditions regarding specification and service without notifying the buyer in advance if no specification or service has been specified and identified in writing before.

Warranty

The warranty terms and conditions in this Standard Terms (the “Warranty”) apply to any instruments, spare parts and services (collectively, the “Products”) purchased by the buyer from **PlexBio** directly and situated in the territory of Taiwan. The Warranty excludes any activities other than calibration, certification and maintenance. **PlexBio** will not provide any Warranty toward the sale and use of the Products outside the territory of Taiwan. The Products distributed outside the territory of Taiwan will be sold “As is”. **PlexBio** will provide the buyer with quality assurance toward the spare parts purchased from **PlexBio** and that are used to maintain **PlexBio** instruments under the same terms and conditions herein in any countries/territories other than Taiwan around the world.

Particularly, the Warranty provided herein excludes any products, software or hardware provided

not by **PlexBio**. If the product is purchased from a **PlexBio**'s distributor, the Warranty shall be provided by the distributor to the buyer in writing directly.

Quality Assurance

Products: Regardless of whether or not the buyer accepts said terms and conditions, for any Products purchased from **PlexBio** directly, **PlexBio** will ensure that the performance of all the Products meets the product specifications provided by **PlexBio** within 12 months from the delivery date on (hereinafter referred to as the quality assurance period). **PlexBio** is entitled to take one of the following actions against any defects found and reported during the quality assurance period: (1) payment refund, or (2) defect repairs or spare parts replacements. The expenses derived from said actions shall be borne by **PlexBio**.

Software: **PlexBio** ensures that all the installed software substantially meets the functions described in the software documentation provided by **PlexBio**. Notwithstanding, **PlexBio** does not guarantee that the software is error-free or may not be attacked by hackers or viruses. The quality assurance period of the software is identical with that of the **PlexBio** product in which the software is installed.

Service: **PlexBio** ensures that, where the buyer finds any nonconformity of related services and notifies **PlexBio** of the same in writing within 30 days after the related services are performed; **PlexBio** shall be obligated to provide necessary services, assistance and consultation to correct the nonconformity.

General clauses: The Warranty and quality assurance provided are subject to the following limitations, including (1) the said quality assurance does not apply to consumable materials, accessories, normal wear and tear, damageable parts and fragile parts; (2) when the buyer asks **PlexBio** to provide quality assurance work beyond normal working hours, **PlexBio** shall be entitled to charge the buyer additional expenses; (3) the said quality assurance does not apply to the following circumstances: accidents, modifications, unfair use, abuse, destruction, or disassembly without authorization, or if the buyer fails to keep or operate in the manner as required, and(or) do unauthorized maintenance, installation or service, and(or) incorporates or integrates any product without permission from **PlexBio** into the **PlexBio** Products, or integrates the **PlexBio** Products into the buyer's environment or products, and(or) uses other software or user interface provided by the buyer/vendor without **PlexBio**'s permission; (4) for the Products sold by **PlexBio** but produced by another manufacturer, the quality assurance provided by **PlexBio** is only valid in the residual period in which the manufacturer provides its quality assurance; (5) once any Products is maintained and repaired by **PlexBio**, the buyer acknowledges that the maintenance

work will not extend the quality assurance period or derive any new quality assurance period. Other than the Warranties and quality assurance explicitly provided in writing in this Standard Terms, **PlexBio** does not provide any other warranties, including but not limited to the warranty toward fitness for a specific purpose, explicitly or implicitly. If the dealer determines, on the basis of its own judgment, that the buyer has misused the Products or failed to follow the instructions to use the Products, the warranty provided by the dealer toward the sale of the Products shall be invalid.

PlexBio shall not be liable for any direct or incidental damage caused by the use of or failure to use the Products, including but not limited to, the loss in the process of operation, shutdown, loss of revenue or profit, loss of the buyer's product or other products, in addition to the liability to be borne by the buyer to the supplier, or by the supplier due to such loss, and the labor or other expenses, damages or losses caused by such Products, including personal injury or loss of property, unless the personal injury or loss of property is caused by the PlexBio's willful conduct or gross negligence.

Maintenance: This maintenance clause applies to Products provided by **PlexBio** within the territory of Taiwan only. The maintenance includes correction, verification, and regular maintenance works only. **PlexBio** does not provide any warranty to the sales outside of Taiwan. The Products sold outside Taiwan shall be labeled "original manufactured" and provided "as-is". In regards of any spare parts placed outside Taiwan and used for the purpose of maintaining the **PlexBio** Products, **PlexBio** shall provide the quality assurance hereunder.

For any Products situated outside the territories of Taiwan for which maintenance is required:

- (i) The buyer shall notify **PlexBio** in writing of the issue of the Products immediately and also provide the details about the issue verified.
- (ii) The buyer shall contact **PlexBio** or the service maintenance engineers trained and qualified by **PlexBio** to evaluate the problems and verify the issues, and might need to bear the related expenses derived from the maintenance or transportation of the Products.
- (iii) The buyer shall return the Products at issue to **PlexBio** or the distributor as per **PlexBio**'s request. **PlexBio** might analyze the Products returned. Upon verifying that no defects exist, **PlexBio** will send the Products back to the buyer and the buyer shall bear the related expenses and freight. Notwithstanding, if **PlexBio** verifies that some issues exist, **PlexBio** will bear the freight. The buyer is not entitled to return the Products without **PlexBio**'s prior written consent.

Notice to User

The use of this instrumentation and its associated software is covered by one or more pending US and foreign patents owned by PlexBio Co., Ltd. The purchase of this instrument and software includes nontransferable rights to use the instrument and software to practice the methods described therein. No general patent or other license of any kind other than this specific right of use from purchase is granted. For further information on purchasing licenses for other applications can be obtained from PlexBio Co., Ltd. 6F-1, No. 351, Yangguang St., Neihu District, Taipei City 11491, Taiwan.

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