

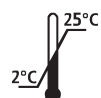


BioGX SARS-CoV-2 Reagents for BD MAX™ System

REF 444213

For Emergency Use Authorization (EUA) Only
For *In Vitro* Diagnostic Use
For use with the BD MAX™ System

P0251(03)
2020-04
English



R_X Only

INTENDED USE

The BioGX SARS-CoV-2 Reagents for BD MAX™ System is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal and oropharyngeal swab samples from individuals 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, to perform moderate and high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swab samples 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 positive 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 BioGX SARS-CoV-2 Reagents for BD MAX System is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR, in vitro diagnostic procedures, and use of the BD MAX System. The BioGX SARS-CoV-2 Reagents for BD MAX™ System is only for use under the Food and Drug Administration's Emergency Use Authorization.

EXPLANATION OF THE TEST

Total nucleic acid (TNA) is isolated and purified using BD MAX™ ExK™ TNA-3 kit from nasopharyngeal and/or oropharyngeal swabs collected in BD Universal Viral Transport System (UVT) or Copan Universal Transport Media System (UTM). Patient sample is transferred to the Sample Buffer Tube (SBT) provided with the BD MAX ExK TNA-3 kit and placed in the BD MAX System. The final eluate is used to rehydrate BioGX SARS-CoV-2 Reagents, which contains all reagents necessary for RT-PCR including primers and probes. This rehydrated master mix is subsequently transferred to a BD MAX PCR cartridge.

The BD SARS-CoV-2 Reagents for BD MAX System utilizes multiplexed primers and probes targeting RNA from the nucleocapsid phosphoprotein gene (N1 and N2 regions) of the SARS-CoV-2 coronavirus, and the human RNase P gene. The primer and probe sets are based on the United States Centers for Disease Control and Prevention (US CDC) assay for specific detection of SARS-CoV-2 by amplifying two unique regions of the N gene (i.e., N1 and N2).

An internal control targeting the human RNase P gene will be co-amplified along with N1 and N2 gene targets (if present) and will serve as an endogenous nucleic acid extraction control present in all properly collected patient samples. This control serves as both an extraction control and an internal amplification control.

PRINCIPLES OF THE PROCEDURE

A combination of lytic and extraction reagents is used to perform cell lysis and DNA/RNA extraction. Nucleic acids released from the target organisms are captured on magnetic affinity beads. The beads, together with the bound nucleic acids, are washed and the nucleic acids are eluted by a combination of heat and pH variation. The final eluate is used to rehydrate BioGX SARS-CoV-2 Reagents, which contains all reagents necessary for RT-PCR including primers and probes. After reconstitution, the BD MAX System dispenses a fixed volume of RT-PCR-ready solution containing extracted nucleic acids into the PCR Cartridge. Microvalves on the cartridge are sealed by the system prior to initiating PCR in order to contain the amplification mixture and thus prevent evaporation and contamination.

The amplified cDNA targets are detected using hydrolysis (TaqMan®) probes, labeled at one end with a fluorescent reporter dye (fluorophore), and at the other end, with a quencher moiety. Probes labeled with different fluorophores are used to detect the target analytes in different optical channels of the BD MAX System. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of target cDNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'–3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the cDNA template. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the optical channels is directly proportional to the quantity of the corresponding probe that is hydrolyzed. The BD MAX System monitors these signals at each cycle of the PCR and interprets the data at the end of the reaction to provide qualitative test results for each analyte.

REAGENTS AND MATERIALS

REF	CONTENTS	QUANTITY
444213	BioGX SARS-CoV-2 Reagents for BD MAX System Lyophilized reagents for multiplexed detection of N1 and RNase P. Each tube is sufficient for a 12.5 µL reaction, sealed in BD MAX 0.3 mL conical tubes	24 tests
	BioGX SARS-CoV-2 Reagents for BD MAX System Lyophilized reagents for multiplexed detection of N2 and RNase P. Each tube is sufficient for a 12.5 µL reaction, sealed in BD MAX 0.3 mL conical tubes	24 tests
	BioGX Rehydration Buffer Sealed in BD MAX 0.3 mL conical tubes. Each tube contains 25 µL of buffer	24 tests

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

- BD MAX System (BD Cat. No. 441916)
- BD MAX Sample Rack (BD Cat. No. 441935, 443550, 443551, 444807, or 444808)
- BD MAX ExK TNA-3 (BD Cat. No. 442827)
- BD MAX PCR Cartridges (BD Cat. No. 437519)
- SARS-CoV-2 Controls
- Copan UTM Collection Kit
- BD UVT Collection Kit
- Vortex Genie 2 (VWR Cat. No. 58815-235 or equivalent)
- Multi-Tube Vortex Mixer (VWR Cat. No. 58816-115 or equivalent)
- Rack compatible with a multi-tube vortexer (e.g., Cryogenic Vial Holder or equivalent)
- Variable Volume Calibrated Pipettor (750 µL volume capable)
- Aerosol resistant micropipette tips
- Disposable gloves, powderless

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use under Emergency Use Authorization only.
- For Prescription Use only.
- 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 positive results to the appropriate public health authorities.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in the CLSI Document M29-A4¹ and in Biosafety in Microbiological and Biomedical Laboratories.² Only personnel proficient in handling infectious materials and the use of BioGX SARS-CoV-2 and BD MAX System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, follow appropriate site procedures.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Protect reagents against heat and humidity. Prolonged exposure to humidity may affect product performance.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or re-use caps, as contamination may occur and compromise test results.

- Check Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Check Unitized Reagent Strips to ensure that all pipette tips are present.
- Proceed with caution when using chemical solutions, as Extraction Tube barcode readability may be altered.
- Good laboratory technique is essential to the proper performance of this assay. Extreme care should be taken to preserve the purity of all materials and reagents.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the BD MAX ExK TNA-3 components, any additional reagents required for testing, and the BD MAX System are not contaminated. Avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges.
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX PCR Cartridge after use. The seals of the BD MAX PCR Cartridges are designed to prevent contamination.
- The laboratory should routinely perform environmental monitoring to minimize the risk of cross-contamination.
- Wear protective clothing and disposable gloves while handling all reagents.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth.
- Do not smoke, drink, chew or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with local, state, provincial and/or federal regulations.
- Consult the BD MAX System User's Manual³ for additional warnings, precautions, and procedures.

STORAGE AND STABILITY

BioGX SARS-CoV-2 Reagents for BD MAX System

BioGX SARS-CoV-2 Reagents for BD MAX System components are provided in sealed pouches and ships at ambient temperature. To protect the product from humidity, immediately re-seal after opening. Stability for open pouch stability for similar products has been established for 1 month at ambient temperature and closed pouch for 6 months at ambient temperature.

INSTRUCTIONS FOR USE

Swab Specimen Collection/Transport

Note: Wear gloves when handling Universal Viral Transport (UVT) or Universal Transport Media (UTM) specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.

1. Nasopharyngeal / oropharyngeal swab specimens should be collected and expressed directly into the BD Universal Viral Transport System or the Copan Universal Transport Media System according to their respective package insert instructions.
2. Transport the UVT/UTM specimen according to the manufacturer's instructions for use.

BD MAX Sample Buffer Tube Preparation

Note: Wear gloves when handling Universal Viral Transport (UVT) or Universal Transport Media (UTM) specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.

Note: If frozen, allow Universal Transport Media (UTM) specimen to come to room temperature before proceeding.

1. Uncap the BD MAX TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM specimen directly into the BD MAX TNA-3 Sample Buffer Tube.
2. Recap the tube with a blue septum cap and vortex or mix by inversion 5 times.
3. Label the BD MAX TNA-3 Sample Buffer Tube with patient information.

Note: Do not obscure the barcodes on the tube. Obscuring the barcode may result in BD MAX System catalog failure and inability to test the sample.

4. Repeat Steps 1 to 3 for each UVT/UTM sample that will be tested on the BD MAX System.
5. Proceed directly with the BD MAX System Operation.

BD MAX System Operation

Note: Refer to the BD MAX System User's Manual³ for detailed instructions (Operation section).

1. Power on the BD MAX System (if not already done) and log in by entering <user name> and <password>.
2. Gloves must be changed before manipulating reagents and cartridges.
3. Remove the required number of Unitized Reagent Strips from the BD MAX ExK TNA-3 kit. Gently tap each Unitized Reagent Strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes. Remove the required number of Extraction Tube(s) from the protective pouch. Remove excess air, and close pouches with the zip seal.
4. From the BioGx SARS-CoV-2 kit, remove the required number of Master Mix Tube(s) and rehydration buffer tubes from the protective pouches. Remove excess air, and close pouch with the zip seal.

5. For each specimen to be tested, place one (1) Unitized Reagent Strip on the BD MAX System Rack, starting with Position 1 of Rack A. Assemble the strip as in Figure 1:

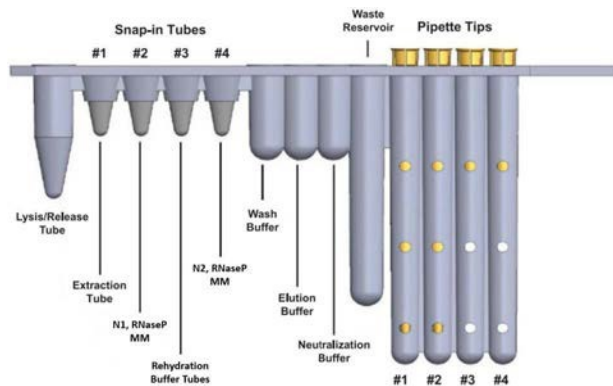


Figure 1: Snap Extraction Tubes and Master Mix Tubes into Unitized Reagent Strips

Note: Failure to add extraction tube and master mix tubes may result in instrument contamination.

Note: A conical snap-in tube is fully seated in the strip when a 'click' is heard. Refer to above for reagent placement in the Unitized Reagent Strip.

- Position 1= Snap the BD MAX TNA-3 Extraction Tube into Position 1.
 - Position 2= Snap the BioGX "N1, RNaseP" lyophilized master mix into Position 2.
 - Position 3= Snap the BioGX Rehydration Buffer tube into Position 3.
 - Position 4= Snap the BioGX "N2, RNaseP" lyophilized master mix into Position 4.
6. Create the User Defined Protocol (UDP) as follows:
 - Navigate to Run > Test Editor tab.
 - Click "Create".
 - Complete each section of the user protocol as outlined in the screen shots below.

Basic Information Section

Parameter	Value	Default	Value Range
Lysis Heat Time	10	10	0 - 30 ± 1 min
Lysis Temperature	60	60	30 - 80 ± 1 °C
Sample Tip Height	1600	1600	1200 - 1600 ± 1 steps
Sample Volume	600	937.5	250 - 950 ± 2.5 µL
Wash Volume	500	500	187.5 - 500 ± 2.5 µL
Neutralization Volume	---	---	---
DNase Heat Time	---	---	---

PCR Setting Section Snap2

Run > Test Editor > Edit > PCR Settings: Snap-In 2 (green)

Test Name: 350093CMAX Extraction Type: ExT TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

PCR Settings

Wavelength	Alias	PCR Gain	Threshold (Cross)	Ct Min	Ct Max
475/520	CoV N1	80	150	0	42
530/565		0	0	0	0
585/630		0	0	0	0
630/665		0	0	0	0
680/715	RNase P	60	100	0	35

Color Compensation

Wavelength	475/520	530/565	585/630	630/665	680/715
475/520	--	0	0	0	0
530/565	0	--	0	0	0
585/630	0	0	--	0	0
630/665	0	0	0	--	0
680/715	0	0	0	0	--

Save Cancel

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Melt Settings Section Snap2

Run > Test Editor > Edit > Melt Settings: Snap-In 2 (green)

Test Name: 350093CMAX Extraction Type: ExT TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

Melt Settings

Wavelength	Alias	Melt Gain	Peak Direction	Sensitivity	Melt Bins
475/520	CoV N1	0	Peak	Medium	Edit Melt Bins
530/565		0	Peak	Medium	Edit Melt Bins
585/630		0	Peak	Medium	Edit Melt Bins
630/665		0	Peak	Medium	Edit Melt Bins
680/715	RNase P	0	Peak	Medium	Edit Melt Bins

Color Compensation

Wavelength	475/520	530/565	585/630	630/665	680/715
475/520	--	0	0	0	0
530/565	0	--	0	0	0
585/630	0	0	--	0	0
630/665	0	0	0	--	0
680/715	0	0	0	0	--

Save Cancel

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Test Steps Section Snap2

Run > Test Editor > Edit > Test Steps: Snap-In 2 (green)

Test Name: 350093CMAX Extraction Type: ExK TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

Test Steps

Step Name: RT

Profile Type: Hold Cycles: 1

Type	Time (s)	Temp (°C)	Detect
	900	43	

Move Add

Step Name: Denaturation

Profile Type: Hold Cycles: 1

Type	Time (s)	Temp (°C)	Detect
	120	99	

Move Add

Step Name: PCR

Profile Type: Temperature Cycles: 45

Type	Time (s)	Temp (°C)	Detect
	5	99	
	16	60	
	25.6	72	<input checked="" type="checkbox"/>

Move Add

Save Cancel

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PCR Setting Section Snap4

Run > Test Editor > Edit > PCR Settings: Snap-In 4 (blue)

Test Name: 350093CMAX Extraction Type: ExK TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

PCR Settings

Channel	Wavelength	Alias	PCR Gain	Threshold (Cross)	Ct Min	Ct Max
	475/520	CoV N2	80	150	0	42
	530/565		0	0	0	0
	585/630		0	0	0	0
	630/665		0	0	0	0
	680/715	RNase P	60	100	0	35

Color Compensation

Excitation Channel	Wavelength	475/520	530/565	585/630	630/665	680/715
	475/520	---	0	0	0	0
	530/565	0	---	0	0	0
	585/630	0	0	---	0	0
	630/665	0	0	0	---	0
	680/715	0	0	0	0	---

Save Cancel

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Melt Settings Section Snap4

Run > Test Editor > Edit > Melt Settings: Snap-In 4 (blue)

Test Name: 350093CMAX Extraction Type: ExK TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

Melt Settings

Channel	Wavelength	Alias	Melt Gain	Peak Direction	Sensitivity	Melt Bins
Channel	475/520	CoV N2	- 0 +	Peak	Medium	Edit Melt Bins
	530/565		- 0 +	Peak	Medium	Edit Melt Bins
	585/630		- 0 +	Peak	Medium	Edit Melt Bins
	630/665		- 0 +	Peak	Medium	Edit Melt Bins
	680/715	RNase P	- 0 +	Peak	Medium	Edit Melt Bins

Color Compensation

Excitation Channel	Wavelength	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	--	- 0 +	- 0 +	- 0 +	- 0 +
	530/565	- 0 +	--	- 0 +	- 0 +	- 0 +
	585/630	- 0 +	- 0 +	--	- 0 +	- 0 +
	630/665	- 0 +	- 0 +	- 0 +	--	- 0 +
	680/715	- 0 +	- 0 +	- 0 +	- 0 +	--

Save Cancel

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Worklist PCR Only Test Editor Inventory

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PCR Setting Section Snap4

Run > Test Editor > Edit > Test Steps: Snap-In 4 (blue)

Test Name: 350093CMAX Extraction Type: ExK TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

Test Steps

Step Name: RT Profile Type: Hold Cycles: 1

Type	Time (s)	Temp (°C)	Detect
	900	43	

Move Add

Step Name: Denaturation Profile Type: Hold Cycles: 1

Type	Time (s)	Temp (°C)	Detect
	120	99	

Move Add

Step Name: PCR Profile Type: 3 - Temperature Cycles: 45

Type	Time (s)	Temp (°C)	Detect
	5	99	
	16	60	
	25.6	72	✓

Move Add

Save Cancel

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Worklist PCR Only Test Editor Inventory

Run Status Results Configuration Reports Maintenance

Result Logic Section

Run > Test Editor > Edit > Result Logic

Test Name: 350093CMAX Extraction Type: Exk TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

Result Logic Steps

Target: CoV N1 Master Mix: Snap-In 2 (green)

Wavelength	Alias	Type	Analyze
475/520	CoV N1	PCR	<input checked="" type="checkbox"/>
680/715	RNase P	PCR	<input checked="" type="checkbox"/>

Edit Logic

Target: CoV N2 Master Mix: Snap-In 4 (blue)

Wavelength	Alias	Type	Analyze
475/520	CoV N2	PCR	<input checked="" type="checkbox"/>
680/715	RNase P	PCR	<input checked="" type="checkbox"/>

Edit Logic

Target: RNaseP1 Master Mix: Snap-In 2 (green)

Wavelength	Alias	Type	Analyze
680/715	RNase P	PCR	<input checked="" type="checkbox"/>
475/520	CoV N1	PCR	<input type="checkbox"/>

Edit Logic

Basic Information

- PCR Settings: Snap-In 2 (green)
- Melt Settings: Snap-In 2 (green)
- Test Steps: Snap-In 2 (green)
- PCR Settings: Snap-In 4 (blue)
- Melt Settings: Snap-In 4 (blue)
- Test Steps: Snap-In 4 (blue)

Result Logic

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Edit Logic

Target: CoV N1

Result	CoV N1 (475/520) PCR	RNase P (680/715) PCR
POS	Valid	Valid
POS	Valid	Invalid
NEG	Invalid	Valid
UNK	Invalid	Invalid

OK

Edit Logic

Target: CoV N2

Result	CoV N2 (475/520) PCR	RNase P (680/715) PCR
POS	Valid	Valid
POS	Valid	Invalid
NEG	Invalid	Valid
UNK	Invalid	Invalid

OK

Edit Logic

Target: RNaseP1

Result	RNase P (680/715) PCR
POS	Valid
NEG	Invalid

OK

Run > Test Editor > Edit > Result Logic

Test Name: 350093CMAX Extraction Type: Exk TNA-3 Master Mix Format: Dual Master Mix: Lyophilized MM with Rehydration Buffer (Type 4)

Result Logic Steps

Target: CoV N2 Master Mix: Snap-In 4 (blue)

Wavelength	Alias	Type	Analyze
475/520	CoV N2	PCR	<input checked="" type="checkbox"/>
680/715	RNase P	PCR	<input checked="" type="checkbox"/>

Edit Logic

Target: RNaseP1 Master Mix: Snap-In 2 (green)

Wavelength	Alias	Type	Analyze
680/715	RNase P	PCR	<input checked="" type="checkbox"/>
475/520	CoV N1	PCR	<input type="checkbox"/>

Edit Logic

Target: RNaseP2 Master Mix: Snap-In 4 (blue)

Wavelength	Alias	Type	Analyze
680/715	RNase P	PCR	<input checked="" type="checkbox"/>
475/520	CoV N2	PCR	<input type="checkbox"/>

Edit Logic

Basic Information

- PCR Settings: Snap-In 2 (green)
- Melt Settings: Snap-In 2 (green)
- Test Steps: Snap-In 2 (green)
- PCR Settings: Snap-In 4 (blue)
- Melt Settings: Snap-In 4 (blue)
- Test Steps: Snap-In 4 (blue)

Result Logic

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Edit Logic

Target: RNaseP2

Result	RNase P (680/715) PCR
POS	Valid
NEG	Invalid

OK

Note: Click on the scroll bar to scroll right.

7. Click <SAVE> after all information has been entered into the Test Editor. The UDP only needs to be created once, and steps 6 and 7 do not need to be repeated for subsequent runs.
8. Click on the Run tab, then Inventory. Enter the kit lot number for the BD MAX ExK TNA-3 (for lot traceability) by either scanning the barcode with the scanner or by manual entry.

Note: Repeat step 8 each time a new kit lot is used.

9. Navigate to the Worklist (RUN > WORKLIST). Using the pull down menu select the UDP previously created in Step 6 (example: 350093CMAX).
10. Enter the Sample Buffer Tube ID, Patient ID and Accession Number (if applicable) into the Worklist, either by scanning the barcode with the scanner or by manual entry.
11. Select the appropriate kit lot number (found on the outer box) from the pull down menu.
12. Repeat Steps 9 to 11 for all remaining Sample Buffer Tubes.
13. Place the Sample Buffer Tubes into the BD MAX System Rack(s) corresponding to the Unitized Reagent Strips previously assembled.

Note: Place the Sample Buffer Tubes into the sample rack with 1D barcode labels facing outward (this makes scanning Sample Buffer Tubes easier during sample login).

14. Place the required number of BD MAX PCR Cartridge(s) into the BD MAX System (refer to Figure2)



Figure 2: Load BD PCR Cartridges

15. Load rack(s) onto the BD MAX System (refer to Figure 3).

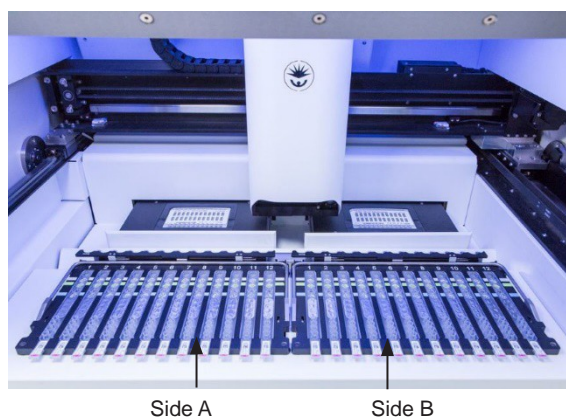


Figure 3: Load Rack(s) onto the BD MAX System

16. Close the BD MAX System lid and click <Start> to begin the processing.

QUALITY CONTROL

Quality control procedures monitor the performance of the assay. Laboratories must establish the number, type, and frequency of testing of control materials according to guidelines or requirements of local, provincial, state, and federal and/or country regulations or accreditation organizations in order to monitor the effectiveness of the entire analytical process. For general Quality Control guidance, the user may wish to refer to CLSI MM3 and EP12.^{1,2}

External Control materials are not provided by BD. External Positive and Negative Controls are not used by the BD MAX System software for the purpose of sample test result interpretation. External Controls are treated as if they were patient samples. (Refer to the table in the Results Interpretation section for the interpretation of External Control assay results.)

It is recommended that one (1) External Positive Control and one (1) External Negative Control be run at least daily until adequate process validation is achieved on the BD MAX System in each laboratory setting. Reduced frequency of control testing should be in accordance with applicable regulations.

The External Positive Control is intended to monitor for substantial reagent failure. The External Negative Control is intended to detect reagent or environmental contamination (or carry-over) by target nucleic acids.

Various types of External Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program.

External Negative Control: A previously characterized sample known to be negative or SBT with 750µL water added.

BD recommends that the External Negative Control be prepared prior to the External Positive Control in order to reduce the potential for contamination as a result of control preparation.

External Positive Control: Commercially available control material from BioGX or other authorized control material may be used.

For the preparation of External Control suspensions, it is recommended that RNA suspensions be prepared in the Sample Buffer Tube (SBT) according to manufacturer's instructions.

All External Controls should yield the expected results (positive for External Positive Control, negative for External Negative Control).

An External Negative Control that yields a positive result is indicative of sample handling and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.

An External Control that yields an Unresolved, Indeterminate or Incomplete test result is indicative of a reagent or a BD MAX System failure. Check the BD MAX System monitor for any error messages. Refer to the System Error Summary section of the BD MAX System User's Manual³ for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new assay kit.

RESULT INTERPRETATION

Results are available on the results tab in the Results window on the BD MAX System monitor. The BD MAX System automatically interprets the test result when the SARS-CoV-2 User Defined Protocol (UDP) is used. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

External Negative and Positive Controls

If the positive or negative control does not exhibit the expected performance as described in the Control Interpretations table below, the assay may have been set up/or executed improperly, or reagent or equipment malfunction could have occurred. In this case, invalidate the run and re-test all samples in that run.

The RNase P gene serves as both a sample extraction control (EC) and an internal amplification control (IAC). In the event that both N1 and N2 region results are negative, an RNase P result must be positive for the BD BioGX SARS-CoV-2 result to be a valid negative result. When either N1 or N2 target result is positive, the RNase P result is ignored.

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Table 1: External Control Interpretations

Control Type	Used to Monitor	Expected Results			
		BioGX "N1, RNase P" master mix		BioGX "N2, RNase P" master mix	
		N1	RNase P	N2	RNase P
Negative Control – SBT with 750µL water	Reagent and/or environmental contamination	UNR*	NEG	UNR*	NEG
Negative Control – Known Negative Sample		NEG	POS	NEG	POS
Extraction Control and RNase P Positive Control	Substantial reagent failure including primer and probe integrity	NEG	POS	NEG	POS
N1 and N2 Positive Control	Substantial reagent failure including primer and probe integrity	POS	NEG	POS	NEG

*UNR is expected result and does not need to be repeated.

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the external positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below lists the expected results. If results are obtained that do not follow these guidelines, re-extract and re-test the sample. If repeat testing yields similar results, collect a fresh sample from the patient for testing.

Table 2: Interpretation of Patient Specimen Results

BioGX "N1, RNase P" master mix		BioGX "N2, RNase P" master mix		Result Interpretation ^{a,b}	Actions
N1 Target	RNase P	N2 Target	RNase P		
+	+/-	+	+/-	Positive	Report as Positive
+	+/-	-	+	Positive	Report as Positive
+	+/-	UNR	-	Positive	Report as Positive
-	+	+	+/-	Positive	Report as Positive
UNR	-	+	+/-	Positive	Report as Positive
UNR	-	-	+	UNR	Repeat Test ^c
-	+	UNR	-	UNR	Repeat Test ^c
UNR	-	UNR	-	UNR	Repeat Test ^c
-	+	-	+	Negative	Report as Negative

^a In the absence of target detection in each of the master mix reactions, the external control (RNase P) must be detected in that master mix reaction in order for the result to be valid.

^b Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

^c Repeat Test by preparing a fresh sample buffer tube from the original primary UVT or UTM sample.

UNRESOLVED, INDETERMINATE, AND INCOMPLETE RESULTS

When an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained a repeat test from the Patient Sample must be performed (see Repeat Test Procedure). If an External Control fails, repeat testing of all specimens conducted on the same day using freshly prepared External Controls (see Quality Control).

Unresolved Result

Unresolved results may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or RNase P amplification. Sample(s) can be repeated from the original Patient Sample. Uncap the BD MAX TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM specimen directly into the BD MAX TNA-3 Sample Buffer Tube. Restart from the BD MAX System Operation section.

Indeterminate Result

Indeterminate results may be obtained in the event that a System failure occurs. Sample(s) can be repeated from the original Patient Sample. Uncap the BD MAX TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM specimen directly into the BD MAX TNA-3 Sample Buffer Tube. Restart from the BD MAX System Operation section.

Incomplete Result

Incomplete results may be obtained in the event that Specimen Preparation or the PCR did not reach its expected time points. Sample(s) can be repeated from the original Patient Sample. Uncap the BD MAX TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 750 µL from the UVT/UTM specimen directly into the BD MAX TNA-3 Sample Buffer Tube. Restart from the BD MAX System Operation section.

External Control Failure

External Controls should yield expected results when tested. If samples have to be repeated due to an incorrect External Control result, the samples should be repeated from the original Patient Sample along with freshly prepared External Controls. Restart from the BD MAX System Operation section.

LIMITATIONS OF THE PROCEDURE

- BioGX SARS-CoV-2 Reagents for BD MAX System has been evaluated only for use in combination with the BD MAX TNA-3 kit and BD MAX System.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swab samples collected in BD Universal Viral Transport System (UVT) or Copan Universal Transport Media System (UTM). Testing of other sample types may result in inaccurate results.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The RNase P endogenous control is included to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- Good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.
- For the BD MAX TNA extraction: Tobramycin at 1.1×10^{-3} g/SBT interferes with the assay. Lower concentrations of Tobramycin have not been evaluated.
- The effect of homeopathic medications for respiratory symptoms on the assay performance was not tested.
- BioGX SARS-CoV-2 Reagent and BD MAX TNA-3 extraction have not been evaluated for patients receiving intranasally administered influenza vaccine.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The BioGX SARS-CoV-2 Reagents for BD MAX System Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations>

To assist clinical laboratories running the BioGX SARS-CoV-2 Reagents for BD MAX System, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories* using the BioGX SARS-CoV-2 Reagents for BD MAX System will include with result reports of the BioGX SARS-CoV-2 for BD MAX System test, 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 the BioGX SARS-CoV-2 Reagents for BD MAX System will perform the BioGX SARS-CoV-2 Reagents for use with the BD MAX System as outlined in the BioGX SARS-CoV-2 Reagents for BD MAX System Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized ancillary reagents, and authorized material required to perform the BioGX SARS-CoV-2 Reagents for BD MAX System test are not permitted.
- Authorized laboratories that receive the BioGX SARS-CoV-2 Reagents for BD MAX System test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the BioGX SARS-CoV-2 Reagents for BD MAX System test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- All laboratory personnel using the BioGX SARS-CoV-2 Reagents for BD MAX System test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Becton, Dickinson and Company, authorized distributors, and authorized laboratories using the BioGX SARS-CoV-2 Reagents for BD MAX System 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.

- Authorized laboratories will collect information on the performance of the BioGX SARS-CoV-2 Reagents for BD MAX System test and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Becton, Dickinson and Company Customer Technical Support 1.800.638.8663 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of BioGX SARS-CoV-2 Reagents for BD MAX System the test of which they become aware.

* For ease of reference, the letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate and high complexity tests" as "authorized laboratories".

NON-CLINICAL PERFORMANCE EVALUATION

Limit of Detection (LoD)

LoD studies determine the lowest detectable concentration of the SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive.

To determine the LoD, quantified genomic viral RNA from SARS-CoV-2, obtained from BEI Resources (Cat. No. NR-52285), was serially diluted into pooled negative nasopharyngeal clinical matrix, a total of 5 concentrations levels, with 2-fold serial dilutions between each level. Confirmation of the estimated LoD was performed with one reagent lot in replicates of 20 prepared in pooled nasopharyngeal swab clinical matrix. The LoD is the lowest concentration (reported as genome equivalents/mL, GE/mL) of genomic RNA from SARS-CoV-2 that can be reproducibly distinguished from negative samples $\geq 95\%$ of the time. The LoD for the assay is 40 GE/mL.

Table 3. LoD determination using genomic RNA from SARS-CoV-2 USA-WA1/2020 strain

RNA from strain	Concentration (GE/mL)	Total Valid Results	Positives			Mean Ct.score			
			SARS- CoV-2	N1	N2	N1	RNase P	N2	RNase P
SARS-CoV-2 USA WA1/2020 (Stock 4.8e+07 GE/mL)	40	20	20	20	20	33.8	22.6	34.3	22.7

Reactivity/ Inclusivity

The nCoV N1 and nCoV N2 primers and probes utilized within the BioGX SARS-CoV-2 Reagents for BD MAX System are identical in sequence to those reported in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The CDC reported an *in silico* analysis of primer and probe sequences within their IFU (CDC-006-0019, Rev 02), and has been copied below for reference:

An alignment was performed with the oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 1, 2020 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel. All the alignments show 100% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one nucleotide mismatch with the N1 forward primer in one deposited sequence.

Cross-Reactivity

The nCoV N1 and nCoV N2 primers and probes utilized within the BioGX SARS-CoV-2 Reagents for BD MAX System are identical in sequence to those reported in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The CDC reported an *in silico* analysis of primer and probe sequences within their IFU (CDC-006-0019, Rev 02), and has been copied below for reference:

BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 10/03/2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

2019-nCoV_N1 Assay:

Probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

2019-nCoV_N2 Assay:

The forward primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is no prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

CLINICAL EVALUATION

The performance of BioGx SARS-CoV-2 Reagents for BD MAX System with retrospective collected nasopharyngeal swab clinical samples was evaluated using 30 individual negative clinical samples and 30 contrived positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection.

Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were handled as described in the package insert of the collection device and stored frozen until use.

Low positive and moderate positive contrived clinical samples were prepared by spiking quantified genomic RNA (SARS-CoV-2 USA-WA1/2020 strain) into individual negative clinical matrix to approximately ~1-2x LoD (20 samples) and ~3-5x LoD (10 samples), respectively.

All low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

Table 4. Clinical evaluation with contrived nasopharyngeal swab samples.

Sample Concentration	Total Valid Results	Agreement with the expected results	BioGX "N1, RNase P" master mix			BioGX "N2, RNase P" master mix		
			% Positive	Mean Ct.score		% Positive	Mean Ct.score	
				N1	RNase P		N2	RNase P
~1-2x LoD	19/20 ^a	19/19	100 (19/19 ^a)	33.8	23.2	100 (19/19 ^a)	33.7	23.0
~3-5x LoD	10	10/10	100 (10/10)	33.1	21.6	100 (10/10)	33.6	21.4
Negative	30	30/30	0 (N/A)	N/A	22.5	0 (N/A)	N/A	22.3

^a During screening one retrospective nasopharyngeal swab clinical sample resulted in an UNR for N1 and as a result was removed from data analysis.

REFERENCES

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (Refer to the latest edition).
2. Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Chosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC)21-1112.
3. BD MAX System User's Manual (refer to the latest revision) BD Life Sciences, Sparks, Maryland 21152 USA.



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$\mu\text{L}/\text{test}$ / $\mu\text{L}/\text{rect}$ / $\mu\text{L}/\text{Test}$ / $\mu\text{L}/\text{εξέταση}$ / $\mu\text{L}/\text{prueba}$ / $\mu\text{L}/\text{teszt}$ / $\mu\text{L}/\text{테스트}$ / mkl/rect / $\mu\text{L}/\text{tyrimas}$ / $\mu\text{L}/\text{pārbaude}$ / $\mu\text{L}/\text{teste}$ / mkl/ananiz / $\mu\text{L}/\text{检测}$



Keep away from light / Избегать от света / Nevystavujte svetlu / Ma ikke udsættes for lys / Vor Licht schützen / Кратко от света / Mantener alejado d' Hoida eemal valgusest / Conserver à l'abri de la lumière / Držati dalje od svetla / Fény nem érheti / Tenere al riparo dalla luce / Қараңғыланған жерде ұста / 빛을 피하 / Laikyti atokiau nuo šaltumos šaltinių / Sargāt no gaismas / Niet blootstellen aan zonlicht / Mā ikke utsettes for lys / Przechowywać z dala od źródła światła / Manter ao ab Ferij de lumină / Хранить в темноте / Uchovávať mimo dosahu svetla / Držite dalje od svetlosti / Får ej utsättas för ljus / Isikitan uzak tutun / Бери ми від дії світла / 1



Hydrogen gas generated / Образуван е водород газ / Možnost úniku plynného vodíku / Frembringer hydrogen gas / Wasserstoffgas erzeugt / Δημιουργία αερίου υδρογόνου / Producción de gas de hidrógeno / Vesinikgaasi tekitatud / Produit de l'hydrogène gazeux / Sadržaj hydrogen vodik / Hidrogeén gáz fejleszt / Produzione di gas idrogeno / ουτερί γαιδα болды / 수소 가스 생성됨 / Išskiria vandenilio dujas / Rodas ūdenradis / Waterstofgas gegenereerd / Hydrogengass generert / Powoduje powstawanie w / Produção de gás de hidrogênio / Generate gaz de hidrogen / Выделение водорода / Vyrobené použitím vodíka / Oslobada se vodonik / Genererad vätegas / Ація цік газу / Реакция з виділенням водню / 产生氢气



Patient ID number / ИД номер на пациента / ID pacienca / Patientens ID-nummer / Patienten-ID / Αριθμός αναγνώρισης ασθενούς / Número de ID del paciente / Patsier / No d'identification du patient / Identifikacijski broj pacijenta / Beteg azonosító száma / Numero ID paziente / Пациенттін идентификациялық нөмірі / 환자 ID 번호 / Pa identifikavimo numeris / Pacienca ID numurs / Identificatienummer van de patiënt / Patientens ID-nummer / Numer ID pacienca / Número da ID do doente / Număr ID pe / Идентификационный номер пациента / Identifikacné číslo pacienca / ID broj pacienca / Patientnummer / Hasta kimlik numarası / Идентификатор пациента / 患者标识



Fragile, Handle with Care / Чупливо, Роботете с необходимото внимание / Кřežká, Při manipulaci postupujte opatrně / Forsigtig, kan gå i stykker / Zerbrechlich, vorsicht handhaben / Εύθραστο, Χειριστείτε το με προσοχή / Frágil, Manipular con cuidado / Öm, käsitlege ettevaatlikult / Fragile, Manipuler avec précaution / Lomljivo, rukujte pažljivo / Törékeny! Óvatosan kezelendő. / Fragile, maneggiare con cura. / Сынғыш, абайлап пайдаланыңыз. / 조심 깨지기 쉬운 유리 / Trapu, elkitas atsargiai / Trausis, atidomiai / Breekbaar, voorzichtig behandelen. / Ľemkľá, hãndler forsitig. / Křucha zavarošć, prizenosć ostrozniě. / Frágil, Manuseie com Cuidado. / Frágil, manijte pažljivo. / Хрупкое! Обращаться с осторожностью. / Křežká, vyžaduje sa opatrná manipulácia. / Lomljivo - rukujte pažljivo. / Bräckligt. Hantera försiktigt. / Kolay Kırılır, Taslıyın. / Тендітна, звертатися з обережністю / 易碎, 小心轻放

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Preparation of External Positive Controls for BioGX SARS-CoV-2 Reagents for BD MAX™ System

Intended Use

The BioGX SARS-CoV-2 Reagents for BD MAX™ System is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal and oropharyngeal swab samples from individuals 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, to perform moderate and high complexity tests.¹

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swab samples 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 positive results to the appropriate public health authorities.¹

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.¹

The BioGX SARS-CoV-2 Reagents for BD MAX System is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR, in vitro diagnostic procedures, and use of the BD MAX System. The BioGX SARS-CoV-2 Reagents for BD MAX™ System is only for use under the Food and Drug Administration's Emergency Use Authorization.¹

Purpose

According to the "Guidance for COVID-19 Testing for CAP-Accredited Laboratories" by the College of American Pathologists, clinical laboratories using EUA assays for COVID-19 testing must perform quality control each of day of patient testing.² External positive and negative controls are samples that act as surrogates for clinical specimens. They are processed like a clinical specimen to monitor the ongoing performance of the entire analytic process in every assay run.

The purpose of this document is to provide clinical laboratories with suggested procedures for preparing External Positive Controls (EPC) for the BioGX SARS-CoV-2 Reagents on the BD MAX System using commercially prepared positive controls and materials.

Table 1. Commercially Prepared Controls for SARS-CoV-2

Manufacturer	SARS-CoV-2 Control	Other Control
BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates ³	SARS-CoV-2 Nucleocapsid Phosphoprotein gene N1 SARS-CoV-2 Nucleocapsid Phosphoprotein gene N2	RNase P
Microbiologics® Helix Elite™ Synthetic RNA Standard ⁴	1044 nucleotide segment of SARS-CoV-2 N (nucleocapsid) gene (N1, N2 and N3)	N/A
Integrated DNA Technologies Positive Control Plasmids ^{5,6}	Complete 2019-nCoV Nucleocapsid gene (N1, N2 and N3)	Portion of the RPP30 single copy gene found in humans

Each suggested procedure for preparing an EPC has been verified by BD. However, the choice of EPC for the BioGX SARS-CoV-2 Reagents for BD MAX System is ultimately the decision of the laboratory, in accordance with applicable local, state, and/or federal regulations, accreditation requirements and the laboratory's standard Quality Control (QC) procedures. Preparation of External Negative Controls (ENC) for the BioGX SARS-CoV-2 Reagents for BD MAX System is outside the scope of this document and should be provided by the laboratory.

A) BioGX SARS-CoV-2 Nucleocapsid N1 and N2 genes and RNase P

The BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates kit contains lyophilized control template beads for the SARS-CoV-2 Nucleocapsid Phosphoprotein gene (N1), SARS-CoV-2 Nucleocapsid Phosphoprotein gene (N2), and RNase P.³ The beads are formulated with quantified RNA (single stranded RNA) at 1×10^5 copies per tube in a lyophilized bead format. Each product package contains 1 pouch of 12 tubes with each tube containing 1 lyophilized control template bead.³ The BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates should be stored at 2-8°C.³

Table 2. Materials Needed for the BioGX External Positive Controls

Material	Part #
BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates	BD # 444214
BD MAX ExK TNA-3 Sample Buffer Tubes	BD # 442827
Nuclease-free water*	Invitrogen # 4387936**

*Alternate diluents e.g., Universal Transport Media (UTM) may be used if validated by the laboratory

**or Equivalent

Preparation of External Positive Controls from BioGX Control Beads

1. To prepare the SARS-CoV-2 N1 and N2 EPC, pipet 650 µL of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
2. Rehydrate the BioGX SARS-CoV-2 N1 and N2 positive control beads individually with 100 µL of nuclease-free water.
3. Pipette the entire volume of the rehydrated N1 and N2 positive controls into the **same** SBT. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the SARS-CoV-2 N1/N2 EPC.

- To prepare the RNase P positive control bead, repeat steps 1-3 above with a **new** BD MAX ExK TNA-3 SBT and the BioGX RNase P positive control beads. The rehydrated RNase P positive control bead is added to the separate SBT.
- Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the tube as the RNase P EPC.
- Proceed with testing the prepared SARS-CoV-2 N1/N2 EPC SBT and the RNase P EPC SBT with the BioGX SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.¹ Each run should include an ENC prepared by the laboratory.

Expected Results

Laboratories should refer to the BioGX SARS-CoV-2 Reagents for BD MAX System Package Insert for full interpretation of external control test results.¹ The expected results are below.

Table 3. Expected results for the SARS-CoV-2 N1/N2 and RNase P External Positive Controls

Specimens	BioGX "N1, RNase P" mastermix		BioGX "N2, RNase P" mastermix		Result Interpretation
	N1	RNase P	N2	RNase P	
SARS CoV-2 N1/N2 EPC	Pos	Neg	Pos	Neg	Positive
RNase P EPC	Neg	Pos	Neg	Pos	Positive

The SARS CoV-2 N1/N2 EPC and RNase P EPC should yield the expected positive results.

B) Microbiologics SARS-CoV-2 Synthetic RNA (N gene targets)

The Microbiologics® Helix Elite™ SARS-CoV-2 synthetic RNA standard is a 1044-nucleotide portion of the SARS-CoV-2 N (nucleocapsid) gene containing the three markers N1, N2 and N3. Each kit includes 1 vial of dried synthetic RNA (concentration: 1×10^9 cps/ μ L), 1 vial of molecular standard water, and a certificate of analysis.⁴ The Helix Elite™ SARS-CoV-2 synthetic RNA standard should be stored at 2-25°C according to the manufacturer's instructions.⁴

Table 4. Materials Needed for the Microbiologics External Positive Control

Material	Part #
Microbiologics® Helix Elite™ Synthetic Standard SARS-CoV-2 Synthetic RNA (N gene Targets)	Microbiologics # HE0060S
BD MAX ExK TNA-3 Sample Buffer Tubes	BD # 442827
Molecular standard or Nuclease-free water*	Invitrogen # 4387936**
1X TE buffer, pH 8.0	Thermo Fisher Scientific # AM9849**
2 mL Tubes	VWR # 10025-756**

*Alternate diluents e.g., Universal Transport Media (UTM) may be used if validated by the laboratory

**or Equivalent

Preparation of External Positive Control from Microbiologics Standard

- To prepare the SARS-CoV-2 Synthetic RNA EPC, add 750 μ L of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
- Rehydrate the lyophilized powder of the Helix Elite™ SARS-CoV-2 Synthetic RNA standard in 100 μ L of nuclease-free water for a concentration stock of 1×10^7 cps/ μ L.
- Dilute the rehydrated SARS-CoV-2 Synthetic RNA by transferring the 100 μ L to 900 μ L of TE buffer for a stock concentration of 1×10^6 cps/ μ L.

4. Prepare serial dilutions of the 1×10^6 cps/ μ L stock in TE buffer to prepare a 100 cps/ μ L stock (See Table 5 for dilution scheme).
5. Pipette 50 μ L of the 100 cps/ μ L diluted stock into the SBT.
6. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the SARS-CoV-2 Synthetic RNA EPC.
7. Proceed with testing the SARS-CoV-2 Synthetic RNA EPC SBT with the BioGX SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.¹ Each run should include an ENC prepared by the laboratory.
8. Aliquot 10 μ L of the remaining 1×10^6 cps/ μ L stock into 2 mL tubes; store the tubes -20°C or colder.

Table 5. Microbiology Control Dilution Scheme from Master Stock

Stock (cps/ μ L)	Dilution Factor	Total Volume of Buffer (μ L)	Volume of Stock to Spike (μ L)	Volume of TE Buffer (μ L)	Dilution Conc (cps/ μ L)
1,000,000	10	1,000	100	900	100,000
100,000	10	1,000	100	900	10,000
10,000	10	1,000	100	900	1,000
1000	10	1,000	100	900	100

Expected Results

Laboratories should refer to the BioGX SARS-CoV-2 Reagents for BD MAX System Package Insert for full interpretation of external control test results.¹ The expected results are below.

Table 6. Expected results for the SARS-CoV-2 Synthetic RNA External Positive Control

Specimens	BioGX "N1, RNase P" mastermix		BioGX "N2, RNase P" mastermix		Result Interpretation
	N1	RNase P	N2	RNase P	
SARS-CoV-2 Synthetic RNA EPC	Pos	Neg	Pos	Neg	Positive

The SARS-CoV-2 Synthetic RNA EPC should yield the expected positive results.

C) IDT 2019-nCoV_N and Hs_RPP30 Positive Control Plasmids

The Integrated DNA Technologies (IDT) 2019-nCoV_N Positive Control plasmid contains the complete nucleocapsid gene from 2019-nCoV (SARS-CoV-2). The Hs_RPP30 Positive Control plasmid contains a portion of the RPP30 gene, a single copy gene present in the human genome.^{5,6} The IDT control plasmids are derived from the CDC nCoV EUA kit. Control Plasmids are delivered at 250 μ L (200,000 copies/ μ L) in IDTE, pH 8.0. Store at -20°C or colder.

Table 7. Materials Needed for the IDT External Positive Controls

Material	Part #
IDT 2019-nCoV_N_Positive Control	IDT # 10006625
IDT Hs_RPP30 Positive Control	IDT # 10006626
ExK TNA-3 Sample Buffer Tubes	BD # 442827
Nuclease-free water*	Invitrogen # 4387936**
1X TE, buffer pH 8.0	Thermo Fisher Scientific # AM9849**
2 mL Tubes	VWR # 10025-756**

*Alternate diluents e.g., Universal Transport Media (UTM) may be used if validated by the laboratory

**or Equivalent

Preparation of External Positive Controls from IDT Control Plasmids

1. To prepare the 2019-nCoV_N EPC add 750 µL of nuclease-free water into a BD MAX ExK TNA-3 Sample Buffer Tube (SBT).
2. Prepare a dilution of the stock IDT 2019-nCoV_N Positive Control plasmid (200,000 copies/µL) in TE buffer (See Table 8 for dilution scheme).
3. Pipette 50 µL of the 200 copies/µL diluted stock into the SBT.
4. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the 2019-nCoV_N EPC.
5. To prepare the Hs_RPP30 EPC, repeat steps 1-3 above with a **new** BD MAX ExK TNA-3 SBT and the IDT Hs_RPP30 Positive Control Plasmid.
6. Close the SBT with a blue septum cap and vortex or mix by inversion 5 times. Label the SBT as the Hs_RPP30 EPC.
7. Proceed with testing the 2019-nCoV_N EPC SBT and the Hs_RPP30 EPC SBT with the BioGX SARS-CoV-2 Reagents for BD MAX System according to instructions in the Package Insert.¹ Each run should include an ENC prepared by the laboratory.

Table 8. IDT 2019-nCoV_N and Hs_RPP30 Plasmid Dilution Scheme

Stock (cps/µL)	Dilution Factor	Total Volume of Buffer (µL)	Volume of Stock to Spike (µL)	Volume of TE buffer (µL)	Dilution Conc (cps/µL)
200,000	10	50	5	45	20,000
20,000	10	100	10	90	2,000
2,000	10	500	50	450	200

Expected Results

Laboratories should refer to the BioGX SARS-CoV-2 Reagents for BD MAX System Package Insert for full interpretation of external control test results.¹ The expected results are below.

Table 9. Expected results for the SARS-CoV-2 Synthetic RNA External Positive Control

Specimens	BioGX "N1, RNase P" mastermix		BioGX "N2, RNase P" mastermix		Result Interpretation
	N1	RNase P	N2	RNase P	
2019-nCoV_N EPC	Pos	Neg	Pos	Neg	Positive
Hs_RPP30 EPC	Neg	Pos	Neg	Pos	Positive

The 2019-nCoV_N EPC and Hs_RPP30 EPC should yield the expected positive results.

Expanding the Protocols

The example protocols described above may be expanded at the laboratory's discretion by increasing the number of each EPC for each run of the BioGX SARS-CoV-2 Reagents for BD MAX System. Please follow the Storage and Stability instructions in the respective Package Inserts of all media and materials used.

IMPORTANT NOTE: Laboratories should follow Good Laboratory Practices and Universal Precautions at all times during preparation and use of external control materials. All materials should be disposed of properly as required by the institution.

Technical Service and Support

BD is committed to providing our customers timely and accurate support. If there are any questions or concerns about this document or the contents, please contact BD Life Sciences – Integrated Diagnostic Solutions Technical Service and Support by dialing 1-800-638-8663 (U.S.).

References

- (1) BioGX SARS-CoV-2 Reagents for BD MAX™ System Package Insert. Becton Dickinson and Company, Sparks, MD. (Latest version).
- (2) College of American Pathologists “Guidance for COVID-19 Testing for CAP-Accredited Laboratories”_
<https://www.cap.org/laboratory-improvement/news-and-updates/guidance-for-covid-19-testing-for-cap-accredited-laboratories>
Accessed March 21, 2020.
- (3) BioGX SARS-CoV-2 & RNase P Synthetic Single- Stranded RNA Control Templates. Package Insert (Latest version)
- (4) Microbiologics® Helix Elite™ Synthetic Standard SARS-CoV-2 Synthetic RNA (N Gene Targets). Package Insert (Latest version)
- (5) Integrated DNA Technologies 2019-nCoV_N_Positive Control Plasmid. Product Description (Latest version)
- (6) Integrated DNA Technologies Hs_RPP30 Positive Control Plasmid. Product Description (Latest version)

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