BIOGENIX

POLICY PROCEDURE FOR EXTRACTION AND RT-PCR REAGENT PERPERATION

	NAME	DESIGNATION	SIGNATURE	DATE
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1. TABLE OF CONTENT

1 REVISION HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

2 REVIEW HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

3 POLICY STATEMENT

The policy defines the process of RNA extraction and RT-qPCR reagent preparation.

4 PURPOSE.

To describe the reagents preparation principles, method, and stability

5 SCOPE

5.1. Describe the procedure of the buffers preparation which include the (Nuclease free water buffer NF, molecular wash -1, and Molecular Wash -2).

5.2. Describe the procedure of the RNA extraction reagent preparation magnetic lysate beads

5.3. Describe the procedure of real time fluorescent q-PCR reagent preparation.

6 DEFINITIONS

Nil

7 ACRONYMS

RT-PCR: Reverse Transcription polymerase chain Reaction

NF: Nuclease Free Water

MW-1: Molecular Wash -1

MW-2: Molecular Wash -2

MLB: Magnetic lysis Beads

UDG: Uracil. DNA. Glycosylase

8 RESPONSIBILITIES

Medical laboratory technologists.

9 PROCEDURE

9.1. Safety and precaution:

9.1.1. You can enter the laboratory after fully dressing in the dressing room (PPE). The specific dressing order is: disposable cap→ N95 mask → protective clothing→ shoe cover→ sleeve →three latex gloves→ face shield

9.1.2. Environmental Control and Safety:

9.1.2.1 The laboratory environmental assessment is qualified;

9.1.2.2. After entering the laboratory, temperature and humidity of the laboratory should be checked and recorded (20 ℃ for freezer while 4 ℃ for refrigerator).

9.1.2.3. Laboratory temperature range: (20 ℃ ~ 25 ℃).

Humidity range: (30% ~ 70%).

9.1.2.4. -20 ℃ freezer temperature range: (-15 ℃ ~ -25 ℃).

refrigerator temperature range: (2 ℃ ~ 8 ℃)

9.2. Principle:

The kit a qualitative in vitro nucleic acid amplification assay to detected the new corona virus using the reverse transcription PCR in specimen

The kit is based on vitro RT-PCR combining fluorescent probing, primers and a sequence –specific fluorescence probes were designed tailored to high conservative region in 2019-nCoV genome. the probes are oligonucleotide attached fluorophore s at the 5'end with FAM as reporter and 3'end with quencher, in the main time, specific

primers and probes were developed vas internal reference with fluorophores VIC /HEX attached at 5' end as reporter. during the PCR procedure, the DNA polymerase cleaves the probes cleaves the probe at the 5'end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA, this cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real –time by PCR detection system, monitoring the fluorescence intensities during real time allows the qualitative detection of 2019-nCoV in specimen

9.3. Buffer MW-1, Buffer MW-2 Reagent Preparation Procedure:

9.3.1. Take out Buffer MW1 and Buffer MW2 from extraction kit.

9.3.2 Prepare a clean measuring cylinder.

9.3.3 Add 360mL 100% Ethanol to each bottle of Buffer MW1; Add 800mL 100% Ethanol to each bottle of Buffer MW2; Vortex and mix well.

9.3.4. Sign "√" on the bottle of the prepared wash buffer, and also mark the batch No. on the bottle in the format such as: MW-Month Day-X（X=1、2、3、4、。。。）, for example: MW-0318-1. Fill the《COVID-19 MW1&MW2 Reagent Preparation Record》

9.4. Magnetic lysis Beads Preparation (MLB):

9.4.1. As showed in the below table, count the number of samples as N , prepare the lysis buffer mix for all samples need to be detected notice to reserve reagent loss. Fill Batch ID in the《COVID-19 Extraction Reagent Preparation Record》, Batch ID format such as: Month Day -E-X（X=1、2、3、4、。。。）, for example: 0318-E1.

NOTE: The lysing solution should be prepared immediately and used within 30 minutes if possible. If preparation is required in advance, the Proteinase K solution should be added before use to avoid prolonged preparation and Proteinase K inactivation

9.4.2. Preparation of Lysing and Binding solution prepare a mix of 160μL of Buffer MLB, 200μL of Absolute Ethanol, 15μL of Proteinase K solution, 15μL of Magnetic Beads M, and 1μL of Enhancer Buffer for each sample to form a Lysis Binding solution. [The lysing solution should be prepared immediately and used within 30 minutes if possible. If preparation is required in advance, the Proteinase K solution should be added before use to avoid prolonged preparation and Proteinase K inactivation].

9.5. Reagent preparation on MGISP-960

Take five 96-deep-well plates (MGI, 1000004644), one for each reagent, label them accordingly, and add the corresponding reagents as shown in Table 2.

Table 2 Sample, Lysis Binding solution, NF Water、MW1、MW2 reagent volume input

Reagent Name	Consumable	Brand	Lot	Reagent volume/ well
Sample	Deep-well plate	MGI	1000004644	160μL
Lysis Binding solution	Deep-well plate	MGI	1000004644	360μL
RNase Free Water	Deep-well plate	MGI	1000004644	60μL
MW1	Deep-well plate	MGI	1000004644	170μL
MW2	Deep-well plate	MGI	1000004644	340μL

9.5.1. MGISP-960 Operation

9.5.1.1 Double-click the icon of MGISP-960 on the desktop. The mode selection interface is displayed, as shown in following figure 1. Select “Real” and click “Create”.

Figure 1. Mode Selection Interface

9.5.1.2. In the Authentication interface, click “User Entry” to enter the initialization interface

Figure 2. Authentication Interface

9.5.1.3. The initialization interface is displayed, as shown in following figure 3.

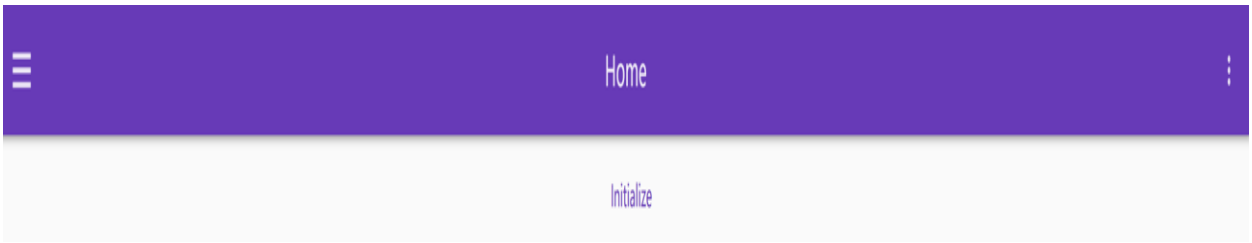

Figure 3. Initialization Interface

9.5.1.4. Click “Initialize”. The initialization takes about 2 min. If Initialize successfully is displayed (as shown in following figure 4, the device is connected successfully, and you can go to the next step

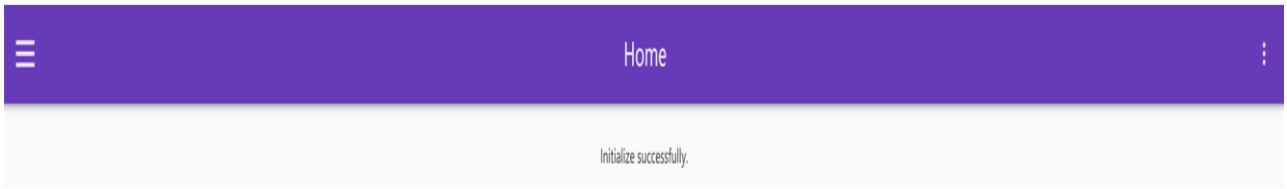

Figure 4. Initialization Successful Interface

Note: If the initialization fails, check whether the power switch is turned on, and whether more than one software program is running. Try to restart the software. If the problem persists, contact MGI technical support.

9.5.1.5. Click the menu button and select “Run Wizard” in the menu. In the Run Wizard interface, click “Solution”, and select which reagent script they want to prepare operation deck arrangement of the first phase is displayed, as shown in Follow the on-screen instructions to place the consumables, samples, and reagents, as

9.5.1.6 Confirm the placement and close the door

9.5.1.7. Click “Run Wizard” to start reagent preparation workflow.

9.5.1.8. It is expected to run 1 h. After the process is finished

9.5.1.9. Perform a post-clean before powering off the device according toMGISP-960 Cleaning instructions.

9.6. QPCR-Mix Reagent Preparation:

9.6.1. Storage and shelf life:

The RT-PCR kit should be stored at temperature lower than -18c in dark. it is stable with shelf –life at 2-8 c for 5 days and at -18 c for 6 months, unpacking kit should avoid repeated thaw-freeze cycle

9.6.1. component of the kit for preparing Q-PCR Mix:

ITEMS	SPECIFICATION	QUANTITY	Description
2019-nCoV Reaction Mix	1ml /Vial	1 Vial	Composed reagent for amplification and probes and primes of target gene and internal reference
2019-nCoV ENZYME Mix	80 µL/Vial	1 Vial	Taq polymerase, Reverse transcriptase and UDG
2019-nCoV Positive control	750 µL/Vial	1 Vial	Mix solution of pseudo-virus with target virus genes and internal reference
2019-nCoV Negative control	750 µL/Vial	1 Vial	DNase free water /RNase Free Water

9.6.7. q-PCR Mix Procedure:

9.6.7.1. Remove the 2019-nCoV Reaction Mix from the kit and leave it at room temperature. After it is completely thawed, vortex and centrifuge briefly for later use. Remove the 2019-nCoV enzyme Mix from the kit, centrifuge briefly and put it on the ice box for later use.

9.6.7.2. To calculate the number of samples N (N = number of samples + 1 positive control + 1 blank control), prepare qPCR-Mix according to the following table, and aliquot 20 μL qPCR-Mix, the 2019-nCoV Reaction Mix and 2019-nCoV enzyme Mix should be immediately put into freezer below -18 ℃.

9.6.7.3. Fill the《COVID-19 qPCR-Mix Reagent Preparation Record》, Batch ID format as: Month Day-Q-X（X=1、2、3、4、。。。）, such as 0318-Q1. Transfer the qPCR-Mix to the sample preparation area 2 with ice box.

9.6.7.4. Fill the QPCR mix transfer record when you transfer the QPCR reagent to extraction room

9.6.7.5. At the end of the shift the endorsement sheet should be fill

Reagent Storage conditions and Effective time after preparation:

Reagent Name	**Status**	**Temperature**	**Effective Time**
NF	96-Deep hole –well plate	Room Temp	24 hr.
MW-1	96-Deep hole –well plate	Room Temp	24 hr.
MW-2	96-Deep hole –well plate	Room Temp	24 hr.
MLB	96-Deep hole –well plate	Room Temp	30 Min
QPCR	96-Deep hole –well plate	-20 °C	4 hr.
QPCR	96-Deep hole –well plate	- 4 °C	2 hr.

10 CROSS REFERENCE

10.1. MGI Easy Nucleic Acid extraction kit user manual

10.2. Real Time Fluorescent RT- PCR kit

10.3. MGISP-960 manual operating user manual

11 RELEVANT DOCUMENTS & RECORDS

11.1. EXTRACTION REAGENT PREPARATION RECORD

11.2. Real time fluorescent RT-PCR for detecting covid -19 reagent requesting and transferring records

11.3 covid -19 quality control summary record -reagent preparation

11.4 . MGISP-960 Daily record