

CE IVD Rx only

PACKIT Central


SARS-CoV-2 (orf 1ab)

Premix Reagent

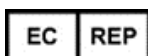
**For Severe Acute Respiratory Coronavirus 2
(orf 1ab) Detection**

User Manual

**Prescription use only
For *in vitro* diagnostic use**

| | |
|---|---|
|  | <p>GeneReach Biotechnology Corp. TEL: 886-4-24639869 FAX: 886-4-24638255 No. 19 Keyuan 2nd Rd., Taiwan Central Science Park, Taichung City 407, Taiwan Web Site: www.genereach.com</p> |
| REF | apcr-123 |

Distributed in United States by GeneReach USA
27 Revere St., Lexington, MA 02420, USA
E-mail: sales@genereachbiotech.com



Mdi Europa GmbH
Langenhagener Str. 71
D-30855 Langenhagen, Germany

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SYMBOLS



Conformité Européenne



in vitro diagnostic medical device



prescription device



Authorized Representative



Contains reagents for 24 reactions



Expiration date



Do not reuse



Do not use if package is damaged



Manufacturer



Catalogue number



Lot number



Temperature limitations




Consult instructions for use

SAFETY INFORMATION

- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Avoid all solutions from contacting with the eyes, skin, and clothes.
- The components in the Extraction Cartridge contain guanidine salt; do not mix them with any solutions that contain bleach or acidic solution.
- When handling samples and performing this assay, users may come in contact with potentially infectious materials. Appropriate biohazard guidelines for working with potentially infectious samples should be followed. It is recommended that the handling of potentially infectious sample be performed in a biological safety cabinet or hood.

For more information, consult the safety data sheets (SDSs). Please contact **GeneReach USA** for SDS if not provided. Below are European Community risk and safety phrases applicable to the components of **POCKIT™ Central SARS-CoV-2 Premix Reagent**.

| |
|---|
| <p>Extraction Cartridge</p>  <p>Danger</p> <p>H314, H318, H400, H410. P264, P280, P303+P362+P353 P305+P351+P338, P273, P310, P391, P501</p> |
|---|

H314: Causes severe skin burns and eye damage.

H318: Causes serious eye damage.

H400: Very toxic to aquatic life.

H410: Very toxic to aquatic life with long lasting effects

P264: Wash hands thoroughly after handling.

P280: Wear protective gloves/protective clothing/eye protection.

P303+P362+P353: IF ON SKIN (or hair): Take off immediately all contaminated

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent

clothing. Rinse skin with water.

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P273: Avoid release to the environment.

P310: Immediately call a POISON CENTER/doctor

P391: Collect spillage

P501: Dispose of contents/container in accordance with local regulations.

INTENDED USE

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent uses the insulated isothermal polymerase chain reaction (iiPCR) technology (Chang *et al.*, 2012; Tsai *et al.*, 2012) and is intended for qualitative detection of severe acute respiratory coronavirus 2 (SARS-CoV-2) RNA in oropharyngeal swab samples from individuals suspected of SARS-CoV-2 infection (COVID-19) during the acute phase 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 high complexity tests, or by similarly qualified non-U.S. laboratories. This assay is intended for *in vitro* diagnostic test purpose.

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 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.

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent is intended for use by trained clinical laboratory personnel specifically on **POCKIT™ Central** system and *in vitro* diagnostic procedures.

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent has to be used with an iiPCR-compatible instrument, **POCKIT™ Central Nucleic Acid Analyzer**.

SUMMARY AND EXPLANATION OF THE TEST

Coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2, a positive-sense single-stranded RNA virus belonging to the *Betacoronavirus* genus and closely related to the SARS-CoV (Gorbalenya *et al.*, 2020). The pathogen is primarily spread via droplets, contact and fomites. Those infected may either be asymptomatic or symptomatic, and some cases can progress into pneumonia and even death (Zou *et al.*, 2020). Nucleic acid amplification tests are recommended to aid for diagnosis of SARS-CoV-2 infection (WHO, 2020).

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent is an iiPCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal swabs from individuals suspected of COVID-19 by their healthcare worker.

PRINCIPLES OF THE PROCEDURE

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent has to be used with a **POCKIT™ Central Nucleic Acid Analyzer** which performs automatic silica-coated magnetic bead-based extraction, reagent reconstitution, iiPCR amplification and detection of target nucleic acid sequences, and data processing and interpretation sequentially.

The **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** is comprised mainly of:

- **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent:** containing lyophilized SARS-CoV-2 (*orf 1ab*) Premix for iiPCR.
- **POCKIT™ Central Cartridge Set (B):** containing reagents and consumables for nucleic acid extraction and liquid transfer.

The **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** is for qualitative duplex detection of SARS-CoV-2 RNA and Internal Control in parallel. The primers and probes target the *orf 1ab* gene of SARS-CoV-2 and a specific sequence of Internal Control. Fluorogenic probe hydrolysis chemistry is used to generate different fluorescent signals, 520 nm and 550 nm, when the specific nucleic acid sequences of SARS-CoV-2 and Internal Control are amplified, respectively. The **POCKIT™ Central Nucleic Acid Analyzer** automatically detects the fluorescence before and at the end of the reaction, processes and interprets the signals based on the built-in algorithm to provide qualitative results.

PRODUCT DESCRIPTION

A. Materials Provided

- **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent**,
24 tests/box

| Component | Contents or Purpose | Amount |
|---|--|--|
| SARS-CoV-2 (<i>orf 1ab</i>) Premix Pack | <ul style="list-style-type: none"> ■ SARS-CoV-2 (<i>orf 1ab</i>) Premix (lyophilized pellet) containing dNTPs, primers, probes, and enzyme for amplification, as well as Internal Control template (non-infectious artificial plasmid). ■ Desiccant. | 3 bags (8 SARS-CoV-2 (<i>orf 1ab</i>) Premix vials and 1 desiccant /bag) |
| Sticker | For labeling use. | 1 piece |
| User Manual | | 1 copy |

- **POCKIT™ Central Cartridge Set (B)**, 24 tests/box

| Component | Description/purposes | Amount |
|----------------------|--|---------------|
| Extraction Cartridge | Preloaded magnetic bead-based nucleic acid extraction reagents. | 24 pieces/box |
| Transfer Cartridge | Consumables for nucleic acid extraction, liquid transfer, and PCR amplification. | 24 pieces/box |
| User Manual | | 1 copy |

B. Materials and Equipment Required, but Not Provided

- **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) P(+)** Control Reagent
- **POCKIT™ Central Nucleic Acid Analyzer**
- Sample collection container
- Vortex mixer
- Centrifuge
- Micropipette, filter tips and microcentrifuge tubes
- Lab coat, gloves and protective goggles

*** Warning: Ensure that all instruments have been checked and calibrated according to the manufacturers' recommendations.**

C. Storage and Stability

- **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** should be stored at 2-8°C and is stable until the expiration date stated on the label of the outside box. Store Premix vials in sealed Premix Pack to avoid hydration of lyophilized components.
- **POCKIT™ Central Cartridge Set (B)** should be stored at 2-30°C and is stable until the expiration date stated on the label of the outside box.

D. Shipping Condition

This assay could be shipped at ambient temperature.

WARNING AND PRECAUTIONS

➤ General Information

- For *in vitro* diagnostic use.
- For prescription use only.
- Please read all warnings, precautions and safety/operation instructions carefully before using this product.
- Check the contents of the assay box upon receiving. If any items are missing or damaged, please contact **GeneReach USA** immediately for a replacement.
- Do not use the assay after the expiration date or with any damaged items as they may lead to poor performance of the test or harms to the user.
- Do not mix components from different kits and/or lots.
- This assay must be used with provided consumables and with

POCKIT™ Central Nucleic Acid Analyzer from GeneReach.

- Protect assay components against heat and humidity. Prolonged exposure to heat and humidity will affect assay performance.
- Positive results are indicative 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.

➤ **Operation**

- Do not modify the operation procedure. Any deviations from the recommended procedures may lead to sub-optimal results.
- The Cartridges are for single use only; do not reuse any consumables.
- The working area should be set away from post PCR amplification area.
- Make sure the operating temperatures during reaction are kept at 15-35°C.
- Proper sample collection, storage and transport are essential for correct results.
- Inclusion of a positive control and a negative control in every run is recommended.
- In case of any spills, please see **TROUBLESHOOTING** section.
- Do not open R-tube(s) after the reaction to prevent any amplicon contamination.
- Follow Good Laboratory Procedures to avoid cross-contamination of the assays.
- Residual nucleic acids on waste materials, such as gloves, cartridges, and used consumables, may lead to contamination of subsequent tests. Dispose them immediately according to local regulation.
- Exposure of components to cleaning agent residues (such as ozone and sodium hypochlorite) or UV may affect the performance of this assay.

LIMITATION OF USE

- Use of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent is limited to personnel who have been trained in the procedure of a molecular diagnostic assay and **POCKIT™ Central Nucleic Acid Analyzer**.
- Performance of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay. Testing of other sample types needs to be validated by users.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors, and/or stage of infection.
- Adding excess amount of sample may interfere with the performance.
- As with all PCR-based tests, extremely low levels of target below the limit of detection of the assay may be detected, but results may not be reproducible.
- Risk of false negative results may occur due to the presence of sequence mutations in the viral target of the test, the presence of inhibitors, technical error, or sample mix-up. Test results may be affected by concurrent antiviral therapy or virus levels in the sample that are below the limit of detection.
- As with any *in vitro* diagnostic test, the results of the assay should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

QUALITY CONTROL

Quality control procedures are intended to monitor assay performance. All control tests should yield the expected results. Any unresolved,

indeterminate, or unexpected results are indicative of false result of the test.

➤ **Internal Control:**

- The Internal Control is included in the Premix Reagent to monitor the performance of the PCR steps.
- The Internal Control is not intended to monitor if target nucleic acid has been lost due to inadequate collection or storage of specimens, or inappropriate pretreatment procedures.

➤ **External Control:**

External Controls should be tested as part of good laboratory practice in accordance with laboratory protocols and accrediting organizations, as applicable. External Control materials are not provided, and are not used by the **POCKIT™ Central** system software for the purpose of interpretation of test result.

- External Controls are treated as if they were patient samples. (Please refer to **DATA INTERPRETATION** section).
- External Positive Control is intended to monitor for substantial reagent failure. Previously characterized positive samples or **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) P(+)** Control Reagent can be used as the external positive control. (Please refer to the **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) P(+)** Control Reagent for instructions on running the positive control).
- External Negative Control is intended to detect reagent or environmental contamination (or carry-over) by target nucleic acids. Viral transport medium can be used as the negative control.

SAMPLE TYPE AND SAMPLE COLLECTION

A. Sample Type

Oropharyngeal swabs

- **Note: Poor sample quality may undermine the test results.**

- **Note: Testing of other sample types needs to be validated by users.**

B. Sample Collection, Handling, and Storage

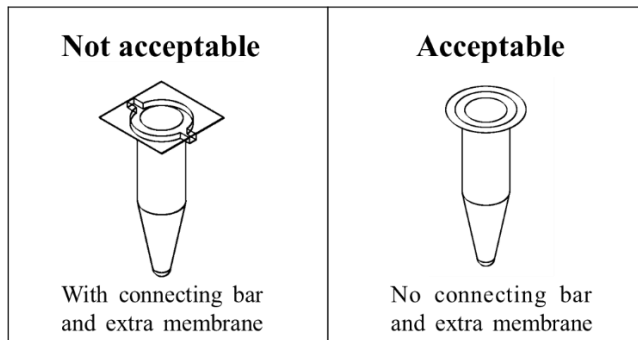
Use only synthetic fiber (such as Nylon) swabs with a plastic or wire shafts to collect oropharyngeal swabs. The swabs should be placed in a transport tube containing viral transport medium (e.g. Copan UTM® 330C).

- **Note: Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inhibit PCR testing.**
- **Note: For specimen collection, handling, transportation and storage, please follow local regulations.**

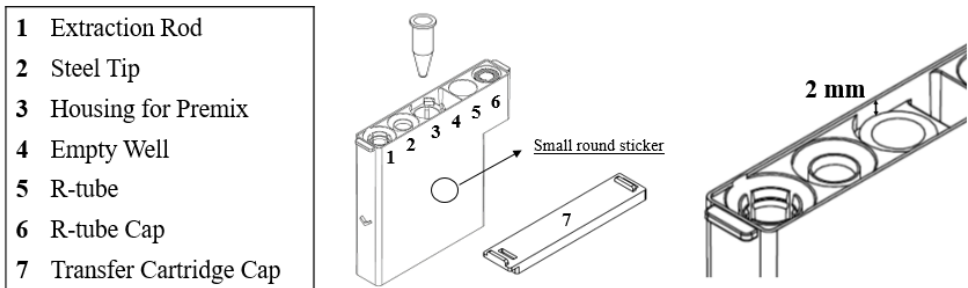
OPERATION PROCEDURE

A. Setup Preparation

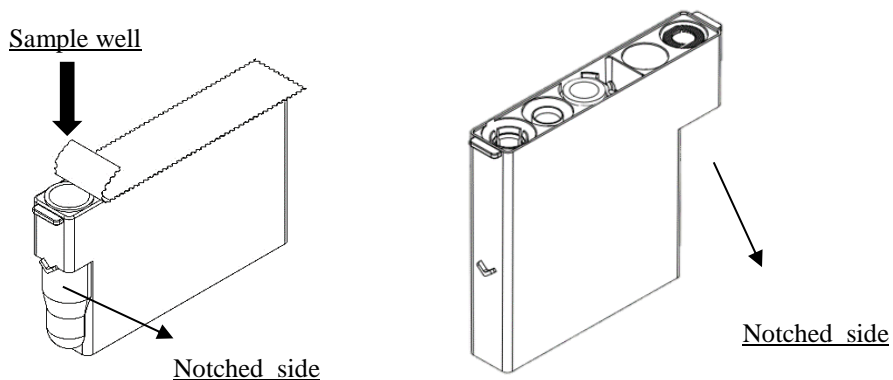
- **Note:** Before preparing the reactions, turn on POCKIT™ Central Nucleic Acid Analyzer for self-testing. Please refer to POCKIT™ Central Nucleic Acid Analyzer’s user manual for programming and setup instructions.
 - **Note:** Perform reaction within 2 hours after the POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent is removed from the aluminum pack.
- 1) For each sample, prepare one set of Extraction Cartridge, Transfer Cartridge and SARS-CoV-2 Premix.
- **Note:** Do not remove the sealing film of the Premix.
 - **Note:** Cut any excessive film and plastics outside the rim of the Premix vial to prevent them from overlapping with neighboring wells in the Transfer Cartridge

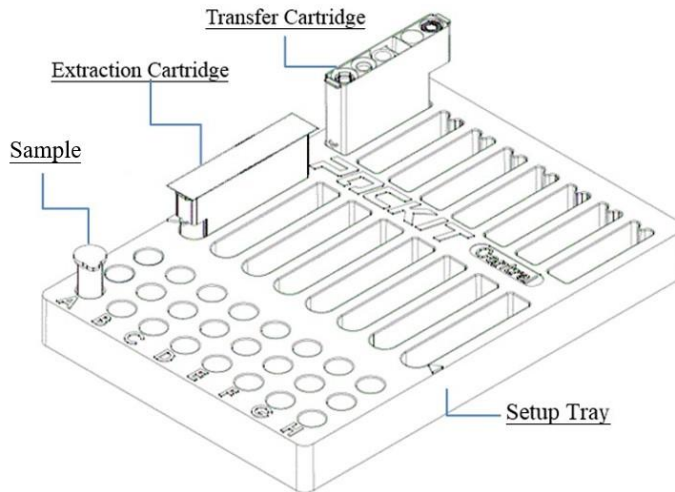


- 2) Remove the Transfer Cartridge Cap, snap the Premix vial into the “Housing for Premix” well of the Transfer Cartridge.



- **Note: Check if all accessories are included in the cartridge.**
 - **Note: Make sure that the lyophilized pellet of SARS-CoV-2 (*orf 1ab*) Premix did not shrink and deliquesce and is collected at the bottom of the Premix vial. When the pellet is not found at the bottom of the vial, swing the cartridge to bring it down.**
 - **Note: Do not throw away the Transfer Cartridge Cap. It will be used to recap the used cartridge after the reaction is finished.**
- 3) Remove the Extraction Cartridge from the aluminum pack.
- **Note: Make sure the liquids are mostly collected at the bottom of Extraction Cartridge in all wells.**
- 4) Turn the notched side of Extraction Cartridge toward you and the notched side of Transfer Cartridge away from you. Place tubes and cartridges on the Setup Tray in the order shown below.





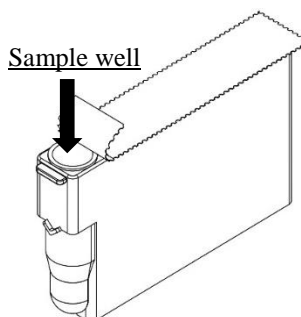
B. Adding sample

Note: Handling and processing of samples should follow local guidelines for processing potentially infectious material.

- 1) Secure the Extraction Cartridge on the Setup Tray with one hand. With the other hand, slowly peel off the sealing aluminum film on sample well of the Extraction Cartridge (first well from the notched side).

■ **Note: Do not remove the rest of the sealing aluminum film from Extraction Cartridge until performing the reaction**

- 2) Load 200 μ l sample to the sample well of the Extraction Cartridge.



C. Cartridge Preparation

- 1) Input the sample ID and the extraction lot number of Extraction

Cartridge from the onscreen keyboard of **POCKIT™ Central Nucleic Acid Analyzer**.

- 2) Secure the Extraction Cartridge on the Setup Tray with one hand. With the other hand, slowly peel off the entire sealing aluminum film on Extraction Cartridge.

■ **Note: Avoid spilling from the cartridge.**



Warning: Make sure to remove any residual sealing film off the cartridge surface.

- 3) Make sure the Transfer Cartridge Cap has been removed.
- 4) Follow the direction on the screen and place loaded Extraction Cartridge to the designated slot.
- 5) Input the Premix Reagent lot number from the onscreen keyboard of **POCKIT™ Central Nucleic Acid Analyzer**.

■ **Note: Please make sure channel “520 nm + 550 nm” is selected.**

- 6) Follow the direction on the screen and place the loaded Transfer Cartridge to the designated slot.

■ **Note: Do not damage the sealing film on the Premix.**

D. Check and Run

- 1) Press **Confirm** to proceed to Run Overview.
- 2) Check if all the information is correct, press **Run** to start the run.

■ **Note: See next section for data interpretation.**

E. After the run is finished

- 1) Put the cap back on the Transfer Cartridges prior to disposal.
- 2) Dispose of all used consumables and waste following local regulations.

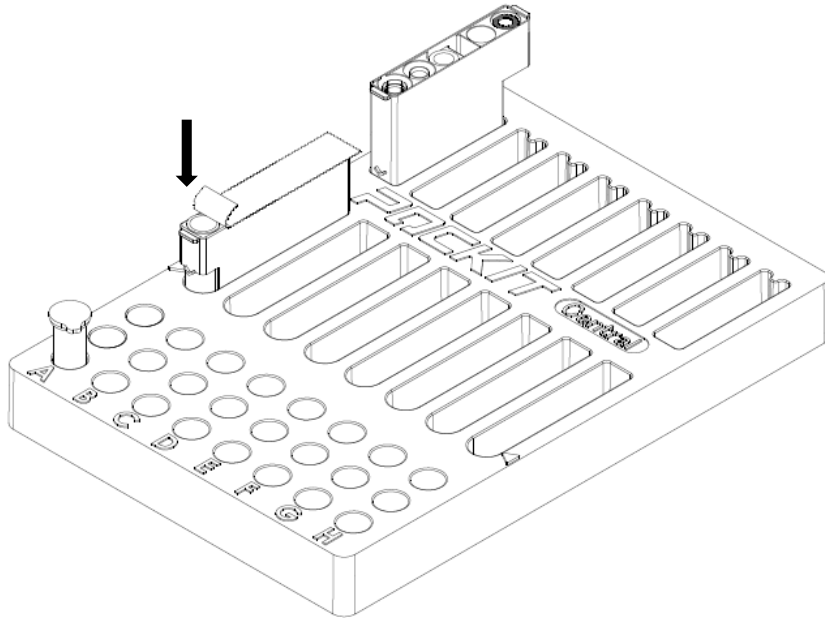
■ **Note: If special disposal handling for steel tips is required**

from local regulation, soak used steel tips with bleach prior to disposal.

OPERATION GUIDE

A. Sample Preparation

- ① Load 200 μ l sample to the sample well of the Extraction Cartridge



B. Cartridge Preparation and Run

②

Slot A Slot B >

Operator: STAFF Reset

Sample ID: Copy

Extraction Lot No.:

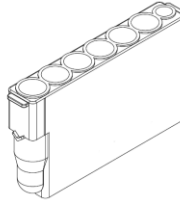
Reagent Lot No.: Channel

Target:

Cancel Run Next Confirm

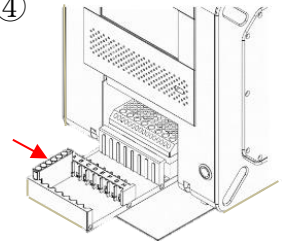
Input Sample ID & Extraction Lot No. using the onscreen keyboard of **POCKIT™ Central** Nucleic Acid Analyzer.

③



Slowly peel off the sealing aluminum film on Extraction Cartridge.

④



Insert the sample-loaded Extraction Cartridge (from step ③) to the selected slot.

⑤

Slot A Slot B >

Operator: STAFF Reset

Sample ID: Copy

Extraction Lot No.:

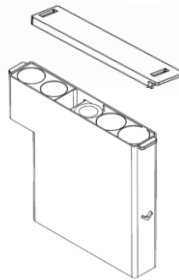
Reagent Lot No.: Channel

Target:

Cancel Run Next Confirm

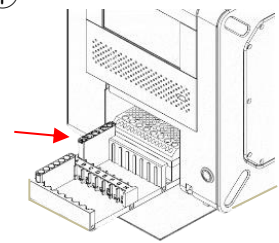
Input the Premix Lot No. using the onscreen keyboard of **POCKIT™ Central** Nucleic Acid Analyzer.

⑥



Remove the cap of Transfer Cartridge and keep it for step ⑩.

⑦



Insert the Transfer Cartridge to the selected slot.

⑧

Reaction In Progress Jul 20, 2017 14:10:10

Current Step Extraction

Time Remaining

85 mins

Abort

Press **Confirm** and **Run** to start reaction. Reaction completes in 1.5 hours.

⑨

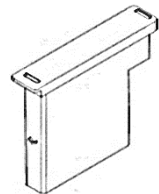
Reaction Overview Operator: SCOTT Report No: 201808170853

| Sample ID | Extraction Lot No. | Reagent Lot No. | Target | CO2 | DOB |
|-----------|--------------------|-----------------|--------|-----|-----|
| A TEST 1 | | | | | |
| B TEST 2 | | | | | |
| C TEST 3 | | | | | |
| D TEST 4 | | | | | |
| E TEST 5 | | | | | |
| F TEST 6 | | | | | |
| G TEST 7 | | | | | |
| H TEST 8 | | | | | |

UV Treatment Is Completed Save to USB End

View results. Please refer to **DATA INTERPRETATION** section for data interpretation.

⑩



Put the cap back on the Transfer Cartridge prior to disposal.

CLEANING

Follow basic laboratory regulations for cleaning procedures for working area:

A. Non-washable and non-movable objects (such as refrigerator)

- 1) Use towel with 10~15% bleach to wipe the surface of object.
- 2) Leave the bleach on the surface for 15 minutes.
- 3) Use a towel with clean water to remove bleach.

Note: For vulnerable objects, wipe them with towel with 75% ethanol to have them dry as fast as possible afterwards.

B. Washable and moveable objects (such as POCKIT™ Central Setup Tray)

- 1) Soak the objects with 10~15% bleach solution for 15 minutes.
- 2) Rinse away the bleach with running water.
- 3) Place the objects on paper towel to dry. If necessary, wipe the surface of object with towels with 75% ethanol to accelerate drying

- **Note: Keep indoor air circulating and wear gloves when cleaning.**
- **Note: Residual bleach may lead to PCR inhibition. Please clean the objects with water thoroughly after bleach treatment.**
- **Note: Follow the protocol of each laboratory to apply UV light for decontamination of the work area. Avoid exposure of specimens and reagents to UV light, since it may affect test results.**

DATA INTERPRETATION

■ Signal interpretation

| Signal | Note |
|--------|-------------------------|
| 520 nm | Target signal |
| 550 nm | Internal Control signal |

*An example of results shown on the monitor.

| Reaction Overview | | Operator:SCOTT | | Report No:201808170853 | | |
|-------------------|--------------------|-----------------|--------|------------------------|-----|--|
| Sample ID | Extraction Lot No. | Reagent Lot No. | Target | 520 | 550 | |
| A | TEST 1 | | | + | + | |
| B | TEST 2 | | | + | + | |
| C | TEST 3 | | | - | + | |
| D | TEST 4 | | | + | + | |
| E | TEST 5 | | | - | + | |
| F | TEST 6 | | | - | + | |
| G | TEST 7 | | | - | + | |
| H | TEST 8 | | | - | + | |

UV Treatment Is Completed

| | 520 nm | 550 nm | Interpretation |
|---------------|--------|--|--|
| Sample | + | + | Positive. SARS-CoV-2 specific RNA detected. |
| | + | - | Positive. SARS-CoV-2 specific RNA detected. |
| | + | ? | Positive. SARS-CoV-2 specific RNA detected. |
| | - | + | SARS-CoV-2 specific RNA are not detected. |
| | - | - | Invalid. Repeat reaction with freshly prepared sample. |
| | ? | + | Invalid. Repeat reaction with freshly prepared sample. |
| | - | ? | Invalid. Repeat reaction with freshly prepared sample. |
| | ? | - | Invalid. Repeat reaction with freshly prepared sample. |
| | ? | ? | Invalid. Repeat reaction with freshly prepared sample. |
| | ! | ! | Warning |
| * | * | Abnormal, signals outside detection range. | |

| | | | |
|--|---|---|---|
| Valid External Positive Control | + | + | Positive. SARS-CoV-2 specific RNA detected. |
| | + | – | Positive. SARS-CoV-2 specific RNA detected. |
| | + | ? | Positive. SARS-CoV-2 specific RNA detected. |
| Valid External Negative Control | – | + | SARS-CoV-2 specific RNA are not detected. |

- **When you see a “ ! ” or “ * ”: Check if all consumables are in their designated wells in the Transfer Cartridge. If yes, repeat reaction from original sample; if not, contact local distributor or GeneReach Technical Service for assistance.**
- **Unresolved results may be obtained due to extremely low levels of target (below the limit of detection in the specimen) in samples, improper sample collection, storage and preparation, or specimen-associated inhibition. Please follow SAMPLE TYPE AND SAMPLE COLLECTION section for sample collection, transportation and storage.**
- **Reaction Failure: Please see TROUBLESHOOTING section.**

TROUBLESHOOTING

| Problems | Possible Causes | Solutions |
|---------------------------------|--|---|
| Contamination | <ul style="list-style-type: none"> ■ Reuse of any consumables. | <ol style="list-style-type: none"> 1. All consumables are for single-use only. Reusing these accessories would cause cross-contamination. 2. Used consumables and waste should be collected and discarded according to local regulations. Do not place them close to the working area to prevent cross-contamination. |
| | <ul style="list-style-type: none"> ■ Contaminated micropipette. | Use filtered tips. |
| | <ul style="list-style-type: none"> ■ Contaminated working area. | <ol style="list-style-type: none"> 1. Start the UV treatment function of POCKIT™ Central Nucleic Acid Analyzer. 2. Consult with a GeneReach technical support representative or local distributor on how to clean up working area. |
| External Control Failure | <ul style="list-style-type: none"> ■ Inadequate storage of positive control | <ol style="list-style-type: none"> 1. Please follow OPERATION PROCEDURE to repeat the test. 2. Consult with a GeneReach technical support representative or local distributor. |
| Reaction Failure | <ul style="list-style-type: none"> ■ Presence of PCR inhibitors | Restart from OPERATION PROCEDURE section. |

| | | |
|----------------------|--------------------------|--|
| | <p>■ Machine failure</p> | <ol style="list-style-type: none"> 1. Check if all consumables are in the designated wells in the Transfer Cartridge. (Please refer to OPERATION PROCEDURE section step B). 2. Consult with a GeneReach technical support representative or local distributor. |
| <p>Spills</p> | <p>■ Operation error</p> | <ol style="list-style-type: none"> 1. Use dry or damp lint-free cloth/wipes to clean the tray of POCKIT™ Central Nucleic Acid Analyzer. Do not leave any liquids on the tray as the electronic parts may be damaged. Gently wipe off any visible stains on the sensors immediately. 2. Start the UV treatment function of POCKIT™ Central Nucleic Acid Analyzer. |

PERFORMANCE EVALUATION

A. Clinical Performance

1) Contrived samples

The clinical performance evaluation of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** was conducted with contrived oropharyngeal (OP) swab samples in viral transport medium. The samples were determined to be negative for SARS-CoV-2 and contrived with *in-vitro* transcribed SARS-CoV-2 RNA fragment at different concentrations. The 35 positive samples tested positive and the 31 negative tested negative by the **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent**. The test results of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** had 100% agreement with the expected results.

| Sample concentration | Target Positive Hit Rate (positive/total, %) | IC Positive Hit Rate (positive/total, %) |
|----------------------|--|--|
| 160 x LoD* | 5/5 (100%) | 5/5 (100%) |
| 16 x LoD | 2/2 (100%) | 2/2 (100%) |
| 1.6 x LoD | 14/14 (100%) | 14/14 (100%) |
| 1 x LoD | 14/14 (100%) | 14/14 (100%) |
| Negative sample | 0/31 (0%) | 31/31 (100%) |
| Positive control | 3/3 (100%) | 3/3 (100%) |
| Negative control | 0/3 (0%) | 0/3 (0%) |

*According to the analytical sensitivity data, the LoD (95% CI) of SARS-CoV-2 IVT RNA was 6×10^4 copies/ml

2) Clinical samples

100 clinical samples were tested by **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent and a reference Real-Time RT-PCR method. Two samples were negative by the reference method and positive by the index method, and one sample was positive by the reference method and negative by the index method. The total agreement is 97.0%, and the Cohen’s kappa coefficient is 0.93 between **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent and reference method, for the detection of SARS-CoV-2 RNA in oropharyngeal swab samples.

| | | Reference method | | |
|---|----------|------------------|-------------------------|-------|
| | | Positive | Negative | Total |
| POCKIT™ Central SARS-CoV-2 (<i>orf 1ab</i>) Premix Reagent | Positive | 30 | 2 | 32 |
| | Negative | 1 | 67 | 68 |
| | Total | 31 | 69 | 100 |
| | | Value | 95% Confidence Interval | |
| Negative agreement (Specificity) | | 97.1% | 91.8%-100% | |
| Positive agreement (Sensitivity) | | 96.8% | 87.0%-100% | |
| Total agreement | | 97.0% | 92.8%~100% | |
| κ | | 0.93 | | |

B. Analytical Performance

1) Analytical Sensitivity : Limit of Detection (LoD)

The analytical sensitivity of the **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent is defined as the concentration (copies/ml) of *in vitro* transcribed SARS-CoV-2 RNA fragment in oropharyngeal swab samples that can be detected with 95% confidence (LoD 95%). The limit of detection (LoD) was 6×10^4 copies/ml.

2) Analytical Reactivity (Inclusivity)

The inclusivity of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent was evaluated by *in silico* analysis of its primers and probes against publicly available sequences of SARS-CoV-2 as of May 04, 2020. 17450 sequences were downloaded from GISAID, NCBI and NMDC nucleotide database and aligned against **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent's primers and probes. 100% homology was found in 17350 sequences (99.43%); 99 sequences (0.57%) had less than 3 mismatches, leading to predicted detectability. Only one (hCoV-19/USA/WA-UW-2105/2020) had 3 mismatches with the assay design and detection reactivity could likely be affected.

3) Analytical Specificity

The cross reactivity of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent was evaluated by wet test and *in silico* analysis. For wet test, a panel of respiratory pathogens or commensal flora found in the respiratory tract were used. Wet testing showed that the **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent did not

cross-react with any of the following pathogens, and no microbial interference to the Internal Control reaction was found (Table.1).

Table.1 Organisms tested for the wet test

| Organisms |
|-------------------------------|
| <i>Escherichia coli</i> |
| <i>Pseudomonas aeruginosa</i> |
| <i>Staphylococcus aureus</i> |
| Influenza A Virus |
| Influenza B Virus |
| Human coronavirus NL63 |
| Human coronavirus 229E |
| Human coronavirus OC43 |
| Adenovirus |
| Respiratory syncytial virus |
| Rhinovirus |
| Parainfluenza type 1 |
| Parainfluenza type 2 |
| Parainfluenza type 3 |

For *in silico* evaluation, each primer and probe of **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** were analyzed against nucleotide sequences in public domains. The results showed no cross reactivity with the organisms listed in the table below (Table.2). **POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent** may cross-react with SARS coronavirus which has been announced to be contained by WHO.

Table.2 Organisms tested by *in silico* analysis

| Organism | Organism |
|----------|----------|
| | |

POCKIT™ Central SARS-CoV-2 (orf 1ab) Premix Reagent

| | |
|--------------------------------------|--|
| Human coronavirus 229E | Cytomegalovirus |
| Human coronavirus OC43 | Enterovirus |
| Human coronavirus NL63 | Epstein Barr Virus |
| Human coronavirus HKU1 | <i>Streptococcus salivarius</i> |
| MERS-coronavirus | <i>Streptococcus pyogenes</i> |
| SARS-coronavirus | <i>Streptococcus pneumoniae</i> |
| Bocavirus | <i>Staphylococcus epidermidis</i> |
| <i>Mycoplasma pneumoniae</i> | <i>Staphylococcus aureus</i> (Protein A producer) |
| <i>Streptococcus</i> | <i>Pseudomonas aeruginosa</i> |
| Influenza A (H1N1) | <i>Neisseria sp.</i> |
| Influenza A (H3N2) | Measles |
| Influenza B | Mumps Virus |
| Human adenovirus, type 1 | Adenovirus type 7 |
| Human Metapneumovirus (hMPV) | <i>Bordetella pertussis</i> |
| Respiratory syncytial virus | <i>Chlamydia pneumoniae</i> |
| Respiratory syncytial virus (type B) | <i>Lactobacillus sp.</i> |
| Rhinovirus | <i>Corynebacterium sp.</i> |
| Human parainfluenza virus type 1 | Influenza B virus (B/Yamagata/16/1988) |
| Human parainfluenza virus type 2 | Influenza B virus (B/Victoria/2/87) |
| Human parainfluenza virus type 3 | <i>Moraxella catarrhalis</i> |
| Human parainfluenza virus type 4 | <i>Mycobacterium tuberculosis</i> (avirulent) |
| <i>Escherichia coli</i> | <i>Neisseria meningitidis</i> |
| Influenza A Virus (H5N1) | <i>Legionella spp</i> |
| Influenza A Virus (H7N9) | Hepatitis A virus (HAV) |
| <i>Lactobacillus sp.</i> | Hepatitis B virus (HBV) |
| Adenovirus C1 | Hepatitis C virus (HCV) |
| Adenovirus 71 | Hepatitis delta virus (HDV) |
| <i>Haemophilus influenzae</i> | Hepatitis E virus (HEV) |
| <i>Pneumocystis jirovecii</i> (PJP) | Human immunodeficiency virus (HIV) |
| <i>Candida albicans</i> | Enterovirus EV68 |

C. Interference Test

POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent was evaluated for potential interference by biological and chemical substances found in oropharyngeal swab specimens. Clinically relevant dose of the potential interference substances were spiked into human oropharyngeal swab samples along with or without *in vitro* transcribed SARS-CoV-2 RNA fragment. The tested substances did not interfere with detection of neither target nor Internal Control by POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent (Table.3).

Table.3 Substances used for the interference test

| Substance |
|-------------------------------|
| Mucin |
| Nasal corticosteroids |
| Oral anesthetic and analgesic |
| Blood |
| Alcohol |

D. Precision

Precision of the POCKIT™ Central SARS-CoV-2 (*orf 1ab*) Premix Reagent was determined as intra-assay variability (Within-Laboratory Precision/Repeatability) and inter-assay variability (Between Laboratory/Lot/Human Reproducibility). Total variability was calculated by combining the two analyses.

The overall percent agreement for the low positive sample category, moderate positive sample category, and negative sample category were all 100% (Table.4).

Table.4 Overall percent agreement of the precision test

| Category | Intra-assay variability | Inter-assay variability |
|--------------------------|---------------------------|---------------------------|
| | Overall Percent Agreement | Overall Percent Agreement |
| Negative Sample | 100% | 100% |
| Low Positive sample | 100% | 100% |
| Moderate Positive sample | 100% | 100% |

E. Carry-over/Cross-contamination

The risks of within-run and between-run carry-over contamination was evaluated by processing specimens with high titers of *in vitro* transcribed SARS-CoV-2 RNA fragment in the **POCKIT™ Central SARS-CoV-2 (*orf 1ab*)** Premix Reagent. Five runs of all positive followed by all negative test were performed for carry-over contamination test, and 5 runs of alternating high titer positive sample with negative reaction for cross contamination test. There were no false positive results, indicating no carry-over or cross-contamination.

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