# WHO Prequalification of In Vitro Diagnostics
# PUBLIC REPORT

## Product: *AccuPower* HIV-1 Quantitative RT-PCR Kit
## WHO reference number: PQDx 0454-140-00

*AccuPower* HIV-1 Quantitative RT-PCR Kit with product code HIV-1111, manufactured by Bioneer Corporation, Rest-of-World, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 10 September 2024.

## Summary of WHO Prequalification Assessment for the *AccuPower* HIV-1 Quantitative RT-PCR Kit

| | Date | Outcome |
|---|---|---|
| **Prequalification listing** | 10 September 2024 | listed |
| **Dossier assessment** | N/A | N/A |
| **Site inspection(s) of quality management system** | 29 May 2023 | MR |
| **Product performance evaluation** | 4th quarter of 2021 and 1st quarter of 2022 | MR |

MR: Meets Requirements
N/A: Not Applicable

**Intended use**

According to the intended use claim from Bioneer Corporation, *"AccuPower HIV-1 Quantitative RT-PCR Kit is an in vitro diagnostic kit designed for quantification of HIV-1 (Human Immunodeficiency virus type1) RNA in human EDTA-plasma samples through real-time polymerase chain reaction (PCR) using ExiStation Universal MDx system. This system includes ExiPrep Dx instrument for automated nucleic acid extraction and the Exicycler for real-time PCR. AccuPower HIV-1 Quantitative RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory makers for the clinical management of HIV-1 infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.*
*HIV-1 Genotype to group M, N, O within range of 8.0 $Log_{10}$ IU/mL (7.74 $Log_{10}$ cp/mL) to 1.70 $Log_{10}$ IU/mL (1.44 $Log_{10}$ cp/mL). This kit is not intended to be used as a screening test for HIV-1 infection in clinical samples including blood and blood products. It is not intended for initial clinical diagnosis of HIV-1 infection, like a HIV-1 screening. The intended user*

*must be a healthcare professional with a knowledge in pathology or bioscience, typically in a hospital and/or clinical laboratory. The use of this kit is only for qualified users who are trained correctly and can safely handle clinical specimens and conduct molecular biological experiments. This kit is not for a lay person, and not for self-testing. The kit is not validated to be tested using PPT EDTA Plasma."*

**Assay description**

According to the claim of assay description from Bioneer Corporation, *"Real-time PCR involves the selective amplification of a dual target sequence (HIV-1 gag-pol gene and LTR region) while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. PCR Reverse transcriptase from the initial RNA promotes the synthesis of the cDNA. After the synthesis, PCR amplification by DNA Polymerase proceeds. The specificity is provided by a pair of specific primers, along with a hydrolysis probe which is also sequence specific. Monitoring amplified product is conducted by labeling the hydrolysis probe with a matched pair of fluorescent dyes (5'-Fluorescent reporter; 3'- Quencher). Due to fluorescence resonance energy transfer (FRET), an intact probe will not emit light. However, upon cleavage by the 5' – 3' exonuclease activity of the DNA polymerase during PCR, the fluorescent reporter molecule will emit a specific wavelength of light within the visible spectrum when cleaved after binding to the amplicon. The kit was designed to maximize reproducibility and ease-of-use by vacuum-drying all reagents for PCR including primers, probes, DNA polymerase, dNTPs and salts by using our proprietary stabilization technology to preserve the full activity of the mixed reagents. The primer-probe set was selected from a pool of primer- probe combinations designed by bioinformatics algorithms to achieve maximized amplification efficiency and to match the thermal cycler program with all of our other AccuPower Diagnostic Kits. So that, this product could be run simultaneously with other kits from AccuPower Diagnostic Kit series."*

**Test kit contents:**

| **Component** | **97 Tests/kit (product code HIV-1111)-Quantity** | |
|---|---|---|
| HIV-1 Premix | 8-well/strip X 12 strips | |
| HIV-1 Standard Positive Controls (SPCs) | HIV-1 SPC[a] (S1) | 1300 µL /tube X 1 tube |
| | HIV-1 SPC (S2) | 1300 µL/tube X 1 tube |
| | HIV-1 SPC (S3) | 1300 µL/tube X 1 tube |
| | HIV-1 SPC (S4) | 1300 µL/tube X 1 tube |
| | HIV-1 SPC (S5) | 1300 µL/tube X 1 tube |
| HIV-1 Positive Controls (LPC and HPC) | HIV-1 LPC[b] | 1300 µL/tube X 3 tubes |
| | HIV-1 HPC[c] | 1300 µL/tube X 3 tubes |
| HIV-1 NTC[d] | 1300 µL/tube X 3 tubes | |
| SL buffer | 1300 µL/tube X 2 tubes | |

| | |
|---|---|
| Optical sealing film | 1 sheet |
| Quick manual | 1 ea |

a: Standard Positive Control b: Low Positive Control c: High Positive Control d: Non-Template Control

**Items required but not provided:**

ExiStation system includes ExiPrep Dx instrument for automated nucleic acid extraction and the Exicycler for real-time PCR.

| **System** | **Instrument** | **Reagent (Extraction)** |
|---|---|---|
| ***ExiStation* (A-2200)** | -*ExiPrep*16 Dx (Cat. No. A-5050)<br>**-***Exicycler* 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060) | **-** *ExiPrep* Dx Viral RNA Kit (K-4473)<br>- *ExiPrep* Dx Viral DNA/RNA Kit (K-4471)<br>- Sample Loading Tube_RNA IPC (Cat. No. KA-3011) |
| *Existation* manager software (Version 1.02.XX) | | |
| ***ExiStation*™ (A-2200-N)** | - *ExiPrep* 16 Dx (Cat. No. A-5050)<br>**-** *Exicycler* 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1) | **-** *ExiPrep* Dx Viral RNA Kit (K-4473)<br>- *ExiPrep* Dx Viral DNA/RNA Kit (K-4471)<br>- Sample Loading Tube_RNA IPC (Cat. No. KA-3011) |
| *Existation* manager software (Version 4.02.XX) | | |
| ***ExiStation* 48 (A-2400)**<br>***ExiStation* 48A (A-2410)** | **-** *ExiPrep* 48 Dx (Cat. No. A-5150)<br>**-** *Exicycler* 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1)<br>- *ExiLT* (Cat.No. A-7100) | **-** *ExiPrep* 48 Viral DNA/RNA Kit (K-4571)<br>**-** *ExiPrep* 48 Viral RNA Kit (K-4573)<br>- *Exiprep* 48 Sample Loading Tube_RNA IPC (KA-4502) |
| *Exiprep* 48 software (Version 1.0.X.X) | | |
| **Etc** | - *ExiSpin* (Cat.No. A-7040) | N/A |
| **General lab- equipment and disposables** | Disposable powder-free gloves<br>Pipette set appropriate volume (1,000 µL, 200 µL, 20 µL pipette)<br>Sterilized pipette tips with filters (1,000 µL, 200 µL, 20 µL tips with filters)<br>1.5 mL or 15 mL conical tubes | |

**Storage:**
The test kit must be stored at -25 to -15 °C.

**Shelf-life upon manufacture:**
12 months.

**Warnings/limitations:**

Refer to the current version of the manufacturer's instructions for use attached to this public report.

**Prioritization for prequalification**
Based on the established eligibility criteria, *AccuPower* HIV-1 Quantitative RT-PCR Kit was given priority for WHO prequalification assessment.

## Product dossier assessment

In accordance with the WHO procedure for abbreviated prequalification assessment, Bioneer Corporation was not required to submit a product dossier for *AccuPower* HIV-1 Quantitative RT-PCR Kit as per the "Instructions for compilation of a product dossier" (PQDx_018 v3).

## Manufacturing site inspection

A manufacturing site inspection of Bioneer Corporation was conducted from 29 to 31 May 2023. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and summarises the assessment findings.

https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports

All published WHOPIRs are with the agreement of the manufacturer.

Based on the site inspection and corrective action plan review, the quality management system for *AccuPower* HIV-1 Quantitative RT-PCR Kit meets WHO prequalification requirements.

## Product performance evaluation

*AccuPower* HIV-1 Quantitative RT-PCR Kit was evaluated by the National Health Laboratory Service, Johannesburg, South Africa on behalf of WHO in the 4th quarter of 2021 and 1st quarter of 2022, according to protocol PQDx_215.

The initial results of this performance evaluation showed a significant bias of 0.37 log10 copies/mL compared to the comparator test (cobas HIV-1 Quantitative nucleic acid test for use on the cobas 6800/8800 Systems (Roche Diagnostics GmbH)), while values in IU/mL showed acceptable agreement with the comparator method. Based on these results and review of calibration data, the manufacturer agreed to review the quantification of the material used to produce the standard curve in copies/mL. As a result, the material used to produce the standard curve was re-quantified and the conversion factor was changed from 0.72 IU/cp to 1.83 IU/cp.

Using the Ct values obtained in the original dataset, the values in copies/mL and IU/mL were re-calculated using the recalibrated standard curve and conversion factor. The results presented below are based on these recalculated values.

### Clinical performance evaluation

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 452 EDTA plasma specimens was used, of which 5 were excluded due to protocol deviation and 20 due to initial user error or failed run and insufficient specimen to repeat. An additional 3 specimens with initial invalid results and insufficient specimen to repeat were included in the calculation of the invalid rate but excluded from performance analyses. The specimens were characterized using the following reference assay: cobas HIV-1 Quantitative nucleic acid test for use on the cobas 6800/8800 Systems (Roche Diagnostics GmbH).

| **Clinical performance characteristics in comparison with an agreed reference standard** | |
|---|---|
| Bias<br>Limits of agreement | 0.105 log10 cp/mL<br>-0.317 to 0.527 cp/mL |
| Invalid rate %<br>(N=427) | 2.6% |
| **Sensitivity and specificity for the detection of virological failure at 1000 cp/mL** | |
| Sensitivity % (95% CI)<br>(N=247) | 98.4 % (95% CI: 95.9 – 99.6) |
| Specificity % (95% CI)<br>(N=86) | 98.8% (95% CI: 93.7 – 100) |
| **Specificity among HIV- negative individuals** | |
| Specificity % (95% CI)<br>(N=91) | 100% (95% CI: 96.0 – 100) |

### Analytical performance evaluation

| **Analytical performance characteristics** | |
|---|---|
| Limit of detection (LoD) | The LoD was estimated at 29 IU/mL (95% CI: 21-58 IU/mL).<br>The LoD claimed by the manufacturer (33.1 IU/mL; 95% CI: 24.5-44.7 IU/mL) was verified. |
| Within-run precision (repeatability) | At $10^3$ cp/ mL, CV% were $\leq$ 1.5%<br>At $10^4$ cp/ mL, CV% were $\leq$ 1.3% |
| Within-laboratory precision (reproducibility) | At $10^3$cp/ mL, CV% were < 2%<br>At $10^4$cp/ mL, CV% were < 2% |
| Linearity | Linearity of the assay was verified for HIV-1 subtypes A, B, C, D, CRF02_AG over a range of viral loads from $10^2$ to $10^6$ cp/mL. |
| Cross-contamination / carry-over | No cross-contamination was observed when high positive and negative specimens were tested together. |

### Operational characteristics and ease of use

This assay requires laboratory equipment and cannot be performed in laboratories with limited facilities or in non-laboratory settings.

The assay was found easy to use by the operators performing the evaluation, who had received a 3-day training by the manufacturer prior to the evaluation.

| **Key operational characteristics** | |
|---|---|
| Specimen type(s) and volume | 400 µL of EDTA plasma |
| Time to result for one run | Approx. 4 hours |
| Operator hands-on time for one run | 70 minutes for preparing specimens, loading reagents, consumable and specimens and sealing and loading strips into the Exicycler. |
| Level of automation | Semi-automated: after extraction in the ExiPrep instrument, strips need to be vortexed and spun within 10 minutes and then loaded onto the Exicycler 96 Real-Time Quantitative Thermal Block. |
| Quality controls | QC (negative, low positive and high positive controls) are provided by the manufacturer. |
| Operating temperature | 15 ~ 30 °C |
| Result display and connectivity | Results are displayed on the connected computer. Results were printed on the standard laboratory printer using a USB containing the reports extracted from the instrument. The results can be exported to the laboratory information system and other health information systems. |
| Power sources | Main power The use of a UPS is recommended, as stable electricity is required. |
| Biosafety *(outside of infectious specimen handling)* | Operators reported biosafety concerns for the user. Specimens, reagents and waste need to be handled according to the manufacturer's instructions. If not, they may cause infections, irritations, corrosions, explosions or maybe carcinogenic. |
| Waste | The volume of liquid was minimal. Waste disposal does not require specific measures in addition to usual laboratory biohazard waste disposal procedures. |
| Calibration | Calibrators are provided by the manufacturer. Calibration must be done when a new lot of extraction or amplification kit is used. |
| Maintenance | Daily maintenance is required. |

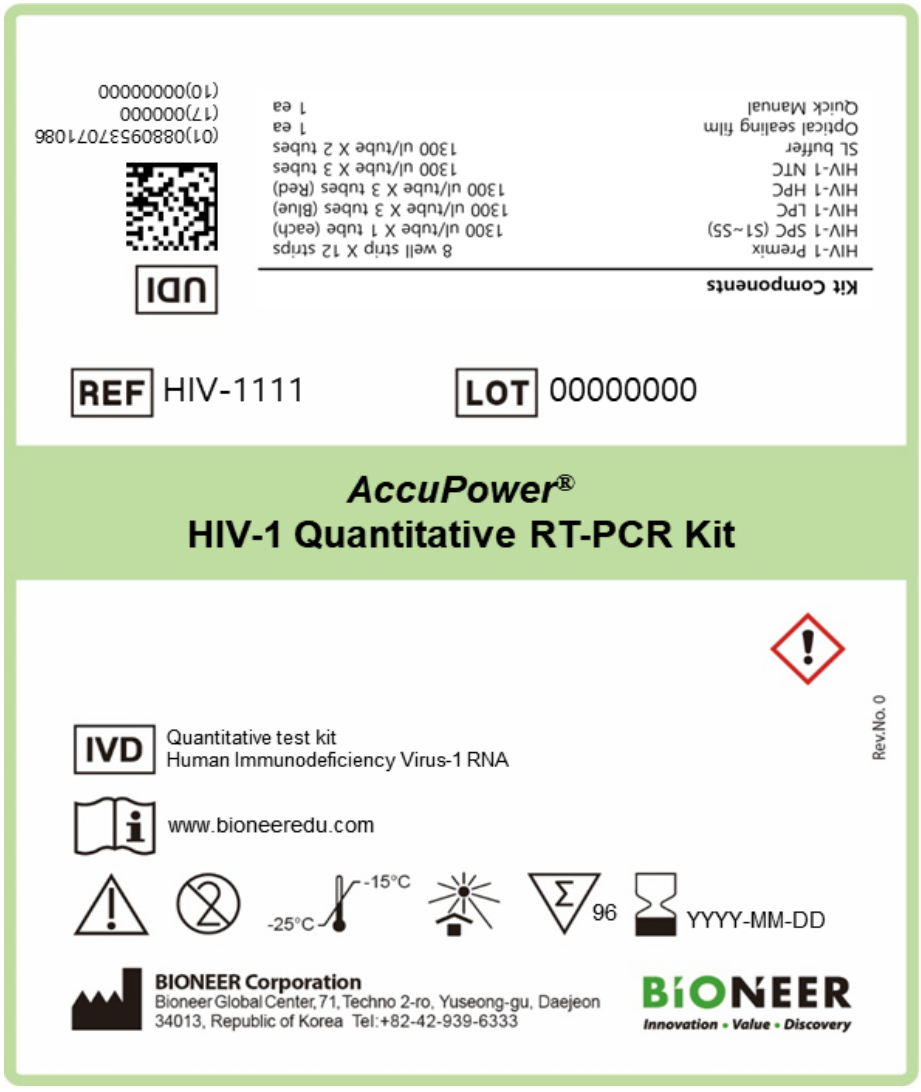
Based on these results, the performance evaluation for *AccuPower* HIV-1 Quantitative RT-PCR Kit meets the WHO prequalification requirements.

## Labelling

1. **Labels**
2. **Instructions for use**
3. **User's Guide**

# 1. Labels

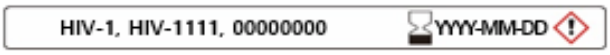
▪ **Label on the Kit box**



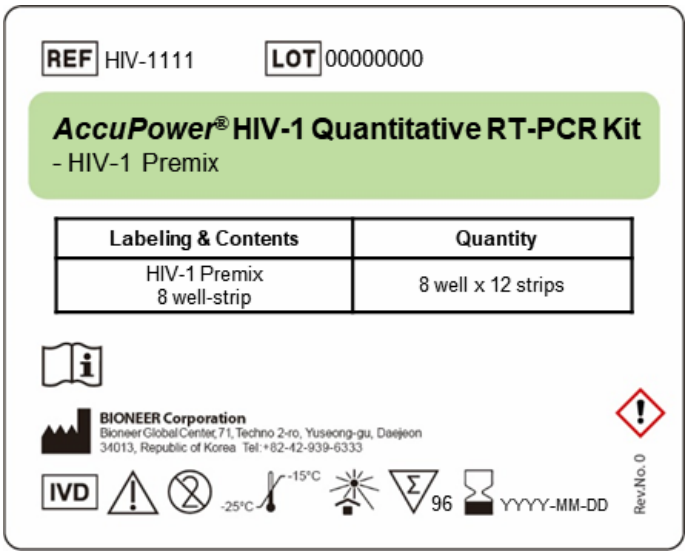
## ▪ Label on the kit component

### 1. Premix

#### 1.1 Primary Label

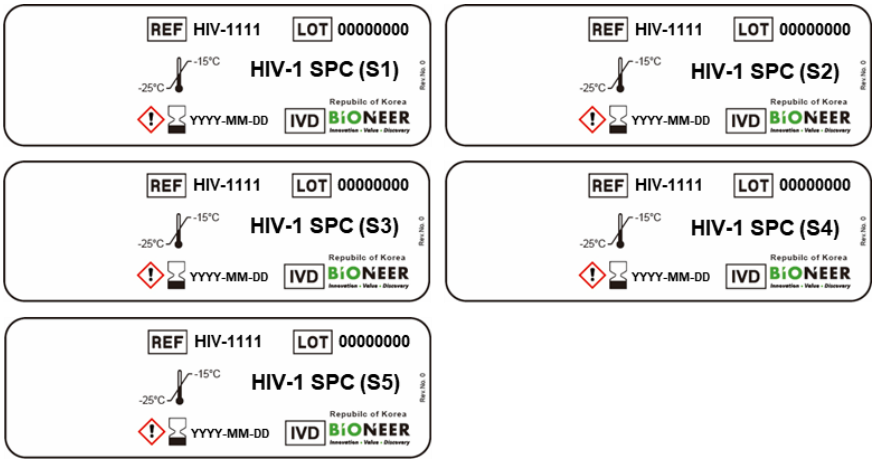



#### 1.2 Secondary Label

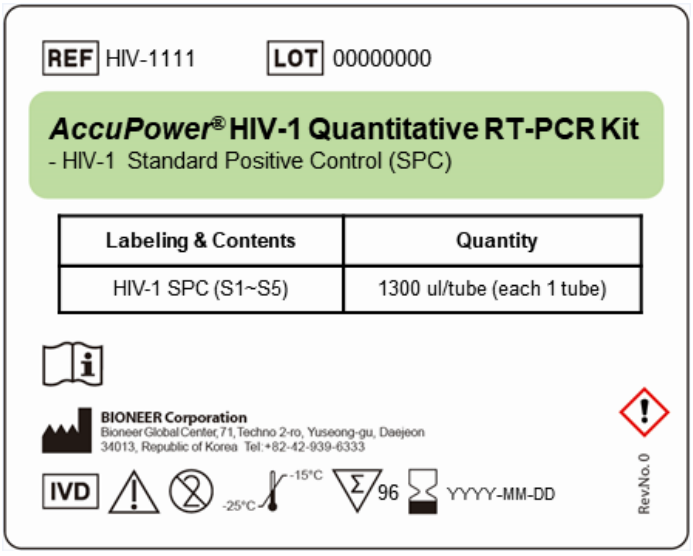


## 2. SPC

### 2.1. Primary Label (Tube Label)



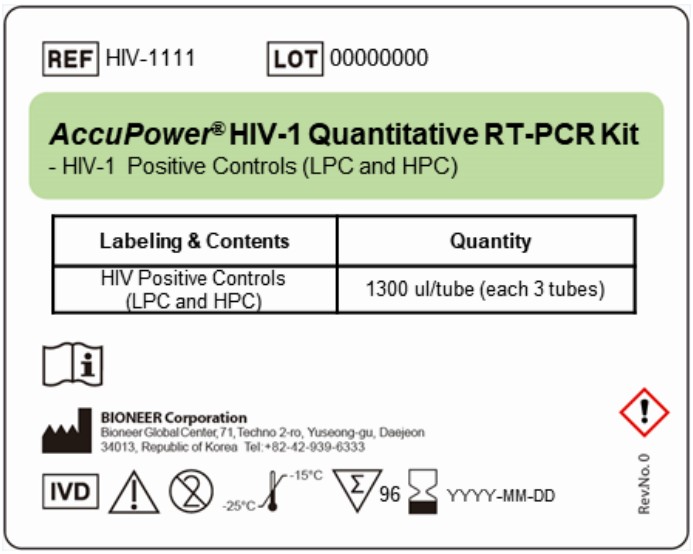

### 2.2. Secondary Label



## 3. LPC and HPC

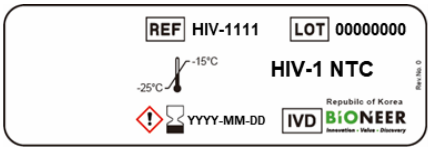
### 3.1 Primary Label (Tube Label)

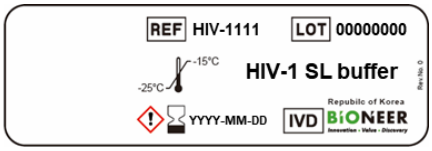

### 3.2 Secondary Label



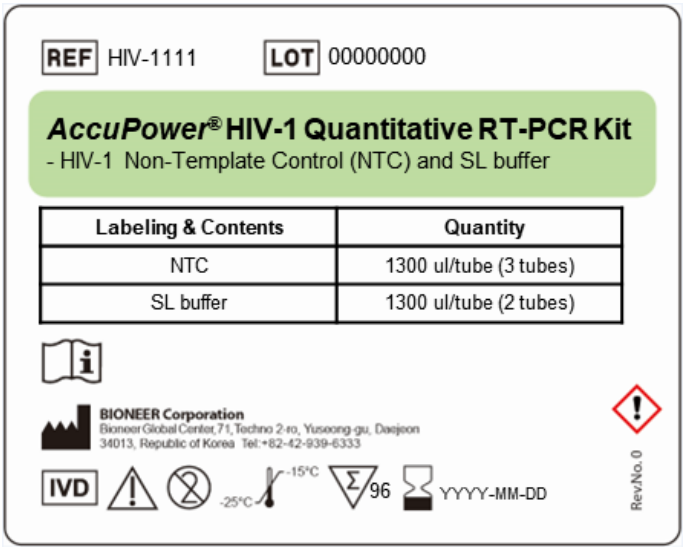
## 4. NTC and SL buffer

### 4.1 Primary Label (Tube Label)




### 4.2 Secondary Label



## 2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

# *AccuPower*® HIV-1 Quantitative RT-PCR Kit



IVD **Quantitative test kit**
**Human Immunodeficiency virus type 1 RNA**

REF **HIV-1111** -25˚C -15˚C Σ 96

www.bioneeredu.com

**For further information, please refer to the User's Guide. The User's Guide can be downloaded by accessing the BIONEER's e-Learning Center (www.bioneeredu.com) and following the path below.**

**Path : Login > SUPPORT > Technical Support > Manual**

After log in, click the [SUPPORT] at the top right of main screen. And click [Manual] in Technical Support to download the User's Guide. Please contact your local BIONEER representative if you need technical support to access the User's Guide.

## 1. INTENDED USE

*AccuPower*® HIV-1 Quantitative RT-PCR Kit is an in vitro diagnostic kit designed for quantification of HIV-1 (Human Immunodeficiency virus type1) RNA in human EDTA-plasma samples through real-time polymerase chain reaction (PCR) using *ExiStation*™ Universal MDx system. This system includes *ExiPrep*™ Dx instrument for automated nucleic acid extraction and the *Exicycler*™ for real-time PCR. *AccuPower*® HIV-1 Quantitative RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory makers for the clinical management of HIV-1 infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.
HIV-1 Genotype to group M, N, O within range of 8.0 $Log_{10}$ IU/mL (7.74 $Log_{10}$ cp/mL) to 1.70 $Log_{10}$ IU/mL (1.44 $Log_{10}$ cp/mL). This kit is not intended to be used as a screening test for HIV-1 infection in clinical samples including blood and blood products. It is not intended for initial clinical diagnosis of HIV-1 infection, like a HIV-1 screening. The intended user must be a healthcare professional with a knowledge in pathology or bioscience, typically in a hospital and/or clinical laboratory. The use of this kit is only for qualified users who are trained correctly and can safely handle clinical specimens and conduct molecular biological experiments. This kit is not for a lay person, and not for self-testing. The kit is not validated to be tested using PPT EDTA Plasma.

## 2. CONTENTS

The Kit is designed to maximize reproducibility and ease-of-use by vacuum-drying all reagents for PCR including primers, probes, DNA polymerase, dNTPs, and salts by using our proprietary stabilization technology to preserve the full activity of the mixed reagents. The primer-probe set was selected from a pool of primer-probe combinations designed by bioinformatics algorithms to achieve maximized amplification efficiency. The accuracy of the assay is guaranteed by Standard Positive Control (SPC) and Positive Controls (LPC and HPC).

**96 tests/Kit**

| Component | Quantity | |
|---|---|---|
| HIV-1 Premix | 8-well/strip X 12 strips | |
| HIV-1 Standard Positive Controls (SPCs) | HIV-1 SPC[a] (S1) | 1300 µL /tube X 1 tube |
| | HIV-1 SPC (S2) | 1300 µL/tube X 1 tube |
| | HIV-1 SPC (S3) | 1300 µL/tube X 1 tube |
| | HIV-1 SPC (S4) | 1300 µL/tube X 1 tube |
| | HIV-1 SPC (S5) | 1300 µL/tube X 1 tube |
| HIV-1 Positive Controls (LPC and HPC) | HIV-1 LPC[b] | 1300 µL/tube X 3 tubes |
| | HIV-1 HPC[c] | 1300 µL/tube X 3 tubes |
| HIV-1 NTC[d] | 1300 µL/tube X 3 tubes | |
| SL buffer | 1300 µL/tube X 2 tubes | |
| Optical sealing film | 1 sheet | |
| Quick manual | 1 ea | |

a: Standard Positive Control b: Low Positive Control c: High Positive Control d: Non-Template Control

## 3. WARNINGS AND PRECAUTIONS

- Please read the *User's Guide* and check the integrity of all tubes and other materials supplied with the kit prior to use.
- *AccuPower*® HIV-1 Quantitative RT-PCR kit is for in vitro diagnostic use only.
- *AccuPower*® HIV-1 Quantitative RT-PCR Kit is intended for use by a qualified clinical diagnostic technician trained to handle and manipulate clinical specimens correctly and appropriately.
- Real-Time PCR using this kit should be performed on *Exicycler*™ 96 Real-Time Quantitative thermal block.
- DO NOT reuse opened reagents, nor mix reagents from different production lots.
- The kit should be stored at -25 ~ -15˚C away from UV/sunlight and is guaranteed stable until the expiration date printed on the label.

### 3.1 Product's Limitations

- The operator should take care of cross contamination.
- Mutations, deletions, and insertions in the target region may cause false positive / negative results.
- The product has been validated only for use with $K_2$ EDTA plasma. Testing of other sample types may lead to inaccurate results.
- Negative test result does not preclude HIV-1 infection. Results from the product should be interpreted in conjunction with clinical presentation and other laboratory makers.
- Before switching from one technology to the next, recommends that users perform method correlation studies in their laboratory to qualify technology differences.
- Reliable results depend on adequate sample collection, transport, storage, and processing.
- Quantitation of HIV-1 RNA depends on the number of virus particles present in a sample and may be affected by sample collection method, patient factors (i.e., age, presence of symptoms), and stage of infection.
- Sample that yields an INVALID result twice may contain the inhibitors, retesting is not recommended. A new sample must be collected if two consecutive INVALID results were obtained from the same specimen.

## 4. PROTOCOL

### 4.1 Preparation of Kit materials and Specimens

- The SPC 1~5, LPC, HPC, and SL buffer should be thawed 15~30˚C. After thawing the SPC 1~5, LPC, HPC, and SL buffer, briefly vortex for 3 seconds and spin-down.
  After thawing the SPC, LPC, HPC, and SL buffer, the test should be performed immediately.

### 4.2 Nucleic acid Extraction

#### 4.2.1 Initialization of the instrument

- ***ExiStation*™ Manager software**

1) Turn on the computer pre-installed with *ExiStation*™ Manager Software.
2) Execute the *ExiStation*™ Manager Software by clicking the icon located on the desktop.
3) Turn on the *ExiPrep*™ 16 Dx by pressing the main power button located at the front of the instrument. Press the "STARTING" image displayed on the LCD to initiate instrument startup.
4) Press the "MISC SET" button on the LCD screen (or the "Load" button on the software). Attach the filter paper onto the Contamination Shield. Attach the prepared Contamination Shield then the Tip Protector in the instrument. Press the "Misc Set" button again.

- ***ExiPrep*™ 48 software**

1) Turn the *ExiPrep*™48 Dx switch on the back of the instrument, and press the POWER button on the front of the instrument for over 1 second.
2) When the initialization of the instrument is completed, the main screen appears on the LCD screen.
3) Log in with the registered ID. When logging in as a guest, it is usually saved data in the folder specified.
4) Touch the Prep icon on the main screen. Enter Prep mode for nucleic acid extraction by touching the *ExiStation*.

#### 4.2.2 Assigning test using software

1) Click the 'Prep' tab on the upper left of the main screen to initiate the nucleic acid extraction process.
2) Click the pull-down arrow for 'Diagnosis Kit 1'. A popup will appear and select 'HIV-1111' from the pull-down menus.
3) After selecting the 'Diagnosis Kit', a popup will appear. Inspect the Buffer Cartridge and mark the used well by clicking on the corresponding location to exclude the used well from sample assignment. Select "OK" to finish.
4) Click the Pull-down arrow for 'Prep Kit'. A popup of the appropriate "Prep Kit" for the selected diagnostic kit will be automatically appear. Select 'Prep Kit' from the pull-down menus.
5) Enter lot number of the diagnostic kit and the prep kit. The program will automatically allocate the NTC and SPC (or PCs) wells.
6) The lot of diagnostic kit and/or extraction kit is new, the program automatically assigns the NTC and SPC 1 to 5. When same lot combination of diagnostic kit and extraction kit are used to previous assay, the standard curve is automatically saved and only 1 LPC (Low Positive Control) and 1 HPC (High Positive Control) are assigned as a positive control.
7) Click the 'Sample ID' column and enter the sample information by using a barcode reader (optional) or a keyboard.

#### 4.2.3 Set-up accessories

- ***ExiStation*™ Manager software**

1) Check that all necessary materials and accessories are present before proceeding.
2) Clean the surface where work will be performed.
3) Remove the shrink-wrap enclosing the both Buffer Cartridges ① and ② then remove the lids in a clean bench.
4) Punch the film with the Hole Puncher according to the layout mapped on the software.
5) Cover Buffer Cartridges ① and ② with the lids after film punching is complete.
6) Place Buffer Cartridges on the setup tray.
7) Take the necessary number of strips of the Diagnostic Kit Tubes from the freezer. Remove the foil covering the tubes.
8) Insert appropriate numbers of Diagnostic Kit Tubes into the Elution Tube Rack. We recommend marking each strip of the diagnostic tubes with the corresponding column number.
9) Fasten the Protection Cover onto the Elution Tube Rack.
10) Place Elution Tube Rack (containing Diagnostic Kit) on the setup tray.
11) Load the appropriate number of disposable filter tips at the disposable tip rack.
12) Place the disposable tip rack and waste tray on the setup tray.
13) Move setup tray to the *ExiPrep*™16 Dx. Open the door and pull the base plate of *ExiPrep*™16 Dx.
14) Place the Buffer Cartridge ② on the heating block of the base plate.
15) Place the Buffer Cartridge ① on the base plate.

16) Place the Elution Tube Rack and Disposable Tip Rack on the base plate.
17) Place the Waste Tray in between the Sample Tube Rack and the Buffer Cartridge ②.

- ***ExiPrep*™ 48 software**

1) Remove the shrink-wrap enclosing both Buffer Cartridges ① and ② within clean bench1.
2) Take the necessary number of Diagnostic Kit tube from the freezer and inset the diagnostic kit tube into the elution tube rack. Remove the covered foil of diagnostic kit tube. Mark each strip of the diagnostic tubes with the corresponding column number.
3) Fasten the protection cover onto the elution tube rack.
4) Open the door of the instrument, remove the setup tray installed inside, and place it on a flat experiment bench.
5) Install Buffer Cartridge ①, ② to the sample quantity on the set-up tray.
6) Install clamp on top of the Buffer Cartridges.
7) Install the Waste Tray.
8) Install the Elution Tube Rack that installed PCR Premix strip and protection cover to the set-up tray.
9) Remove the cover of the disposable tip rack and install it on the setup tray.
10) Install the 8-hole punch.

### 4.2.4 Nucleic acid Extraction

1) Prepare of clinical samples, sample loading tubes and controls in BSC. Clean the BSC(Class Ⅱ,Ⅲ) on which the nucleic acid extraction preparation will be performed.
2) Take out the RNA IPC Sample Loading Tubes from the packaging, mark it with sample name and insert them into the rack.
3) Take the original clinical sample containers and controls (NTC and SPC) and pipette into the RNA IPC Sample Loading Tubes by following step 4)~8).
4) Add 400 µL of NTC into a tube assigned as NTC. (supplied with the *AccuPower*® Diagnostic Kit)
5) Additionally add 400 µL SPC 1~5 into the appropriate SPC wells. (supplied with the *AccuPower*® Diagnostic Kit)
6) Move the filled Sample Tube into the Sample Tube Rack.
7) Uncap clinical sample container and pipette 400 µL sample into RNA IPC Sample Loading Tube. Move the RNA IPC Sample Loading Tube into Sample Tube Rack when it is filled with sample.
8) Repeat the sample loading steps individually until all samples are loaded.
9) Remove the waste tray on the base plate (Only applicable to *ExiStation*™ manager software).
10) Load the Sample Tube rack on the *ExiPrep*™ Dx base plate.
11) Place the Waste Tray in between the Sample Tube Rack and the Buffer Cartridge ②.
12) All materials are loaded and remove the lids from Buffer Cartridges.
13) Check whether all accessories are loaded properly.
14) Slide the base plate in and close the door of the *ExiPrep*™ Dx.
15) click the 'RUN(▶)' icon of the software (In the *ExiPrep*™ 48 Software, click the 'Apply Run' icon).

### 4.3 Real-Time PCR

1) In order to logically process the samples in a single real-time PCR run without confusing the order or identify of samples, please follow the instructions below.
2) After the nucleic acid extraction process is finished, the cooling block is automatically turned off.
3) Open the door of the *ExiPrep*™ Dx after the nucleic acid extraction process is complete, and then remove the Elution Tube Rack.
4) Use the Protection Cover Separation Tool to remove the Protection Cover as follows.
5) Place the Elution Tube Rack from *ExiPrep*™ Dx on the Protection Cover Separation Tool carefully.
6) Firmly hold down Protection Cover and Separation Tool with on hand. Rotate the lever in a clockwise 180˚ with the other hand.
7) Press down both sides of Separation Tool. This action will push Protection Cover upwards so that Elution Tube Rack can be removed with ease.
8) Taking care not to flip the orientation of the tubes, Place the Elution Tube Rack on the PCR Preparation Plate with the corresponding *ExiPrep*™ number.
9) Seal the Diagnostic tubes with the adhesive Optical sealing film.
10) Right before the PCR reaction, completely mix the tube contents using *ExiSpin*™. (*ExiSpin* ™ parameters: 2500rpm for 1 sec., Hard vortex for 20 sec., 20 cycles).
11) While the *ExiSpin*™ is operating, turn on the *Exicycler*™ 96.
12) Turn the Standby Power Switch, located at the rear of the instrument ON. The front ring-LED status light should turn on Blue.
13) Press the front Operation Power Switch for 3 seconds. A brief self-test sequence will initiate. If the self-test passes, the front ring-LED will blink GREEN with a short beep.
14) Click 'Assign PCR' tab (In the *ExiPrep*™ 48 software, click the 'Assign' icon) and check the box of each 'Prep Work List' to assign PCR position.
15) Push the Door Switch for 2 seconds to slide the 96-well thermal block out. Insert the reaction tubes in their pre-determined locations. After sample loading is complete, push the Door Switch for 2 seconds to close the door.
16) Place the mixed premix tubes into the assigned well position of *Exicycler*™ 96 right after the *ExiSpin*™ cycling is complete. For detailed operation instructions of *Exicycler*™ 96, *ExiStation*™ software, see the relevant User's Guide.
17) Select 'Assign PCR' tab and confirm the assigned 'Prep Work List'. After the 'Prep' process, 'Current Step' will be presented as 'Prep End' and the upper status bar will be changed to 'Ready to PCR'. Initiate PCR run by clicking the activated 'PCR Start' icon at the bottom right of the window (In the *ExiPrep*™48 software, click the 'Run PCR' icon).
18) A popup will appear prompting the user to enter a Work List Name. Click 'OK' after entering a name to generate a Work List for Real-Time PCR.
19) After entering the Work List Name, 'PCR' tab will be activated and the *Exicycler*™ 96 will automatically initiate PCR run.
20) Remove all consumables and components, starting with the Buffer Cartridges and various rack from the instrument and discard all liquids and consumables in their appropriate containers.
21) Push the Base Plate in, shut the instrument door and initiate UV sterilization by clicking 'UV ON' on the control panel.

### 4.4 Data Analysis

1) After the PCR run is finished, select 'RESULT' tab to check the results of each sample.
2) The result data files are saved automatically on this path.

| Software | Result data file path |
|---|---|
| *ExiStation*™ manager software | C:\ExiStation_Data\user\GUEST\ WorkList\'relevant data file name' folder |
| *Exiprep*™48 software | *ExiPrep*™ 48 software > SET UP > Data > WorkList > relevant data file |

## 5. ORDERING INFORMATION AND RELATED PRODUCTS

| Cat.no. | Description |
|---|---|
| HIV-1111 | *AccuPower*® HIV-1 Quantitative RT-PCR Kit |
| K-4471 | *ExiPrep*™ Dx Viral DNA/RNA Kit |
| K-4473 | *ExiPrep*™ Dx Viral RNA Kit |
| K-4571 | *ExiPrep*™ 48 Viral DNA/RNA Kit |
| K-4573 | *ExiPrep*™ 48 Viral RNA Kit |

## 6. TECHNICAL SUPPORT & A/S

- If any issues are discovered relating to compromise in product quality and test results, immediately contact BIONEER's Customer Service Center (ds@bioneer.com).
- For the quick and precise response, send us the information about the assay, attaching the applicable test data.

| Category | Information |
|---|---|
| Nucleic Acid Purification | Instrument (Manufacturer / serial No.)<br>Kit (Manufacturer / Lot No.) |
| Real-Time PCR | Instrument (Serial No.)<br>Kit (Cat. No / Lot No.) |
| Applicable ex3 file and log file | |

## 7. NOTICE & TRADEMARK

BIONEER Corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications, or product descriptions at any time without notice. All information provided here is subject to change without notice.

*AccuPower*® is a trademark of BIONEER Corporation.

## 8. REVISION SUMMARY

| Version | Date | Summary |
|---|---|---|
| 4.7 | 2024-06-13 | Added the website URL for electronic instruction for use<br>Updated of 1. INTENDED USE<br>Added 3.1 Product's Limitation<br>Updated 4.1 Preparation of Kit materials and Specimens |

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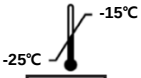
# 3. User's Guide

USER'S GUIDE

Innovation • Value • Discovery

# *AccuPower*®
# HIV-1 Quantitative RT-PCR Kit




REF HIV-1111

IVD Quantitative test kit
Human immunodeficiency virus-1 RNA

BIONEER
Innovation • Value • Discovery

# *AccuPower*® HIV-1 Quantitative RT-PCR Kit

## User's Guide


**Version No.: 3.9 (2024-06-13)**

**Please read all the information in booklet before using the unit**

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## Safety warning and Precaution

Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

Please read the User's Guide and check the integrity of all tubes, tips and other materials supplied with this kit prior to use.

Before, during and after use of this kit as described in this User's Guide, all potentially hazardous materials (i.e. materials that may have come in contact with clinical samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/ government in which this product is being used. Adhere to general clinical laboratory safety procedures during the experiment.

## Warranty and Liability

All BIONEER products are manufactured and tested under strict quality control protocols. BIONEER guarantees the quality of all directly manufactured products until the expiration date printed on the label. If any issues are discovered relating to compromise in product quality, immediately contact BIONEER's Customer Service Center (ds@bioneer.com).

BIONEER does not assume liability for misuse of the product, i.e. usage of the product for any purposes other than its intended purpose as described in the appropriate and applicable User's Guide. BIONEER assumes liability under the condition that the user discloses all information related to the problem to BIONEER in written form within 30 days of occurrence.

## Legal Disclaimer

Some applications that may be performed with this kit may infringe upon existing patents in certain countries. The purchase of this kit does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on country and application. BIONEER does not condone nor recommend the unlicensed use of a patented application.

The use of the kit is only for qualified and well-trained users in handling of clinical specimens and molecular biological experiments. After testing, all wastes should be processed with the fulfillment of the regulation of the country.

## Trademark



## Copyright



## Notice

# Contents

## 1. INTENDED USE

*AccuPower*® HIV-1 Quantitative RT-PCR Kit is an in vitro diagnostic kit designed for quantification of HIV-1 (Human Immunodeficiency virus type1) RNA in human EDTA-plasma samples through real-time polymerase chain reaction (PCR) using *ExiStation*™ Universal MDx system. This system includes *ExiPrep*™ Dx instrument for automated nucleic acid extraction and the *Exicycler*™ for real-time PCR.

*AccuPower*® HIV-1 Quantitative RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory makers for the clinical management of HIV-1 infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

HIV-1 Genotype to group M, N, O within range of 8.0 $Log_{10}$ IU/mL (7.74 $Log_{10}$ cp/mL) to 1.70 $Log_{10}$ IU/mL (1.44 $Log_{10}$ cp/mL). This kit is not intended to be used as a screening test for HIV-1 infection in clinical samples including blood and blood products. It is not intended for initial clinical diagnosis of HIV-1 infection, like a HIV-1 screening. The intended user must be a healthcare professional with a knowledge in pathology or bioscience, typically in a hospital and/or clinical laboratory. The use of this kit is only for qualified users who are trained correctly and can safely handle clinical specimens and conduct molecular biological experiments. This kit is not for a lay person, and not for self-testing. The kit is not validated to be tested using PPT EDTA Plasma.

## 2. INTRODUCTION

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS). This virus is passed from one person to another through blood-to-blood and sexual contact. In addition, infected pregnant women can pass HIV to their baby during pregnancy or delivery, as well as through breast-feeding.[(1)~(5)]

HIV-1 infection has a 3 stage that is an acute HIV infection, chronic HIV infection and AIDS, acute HIV infection is earliest stage of infection that occurs at a median of 12 days after exposure in 40%–70% of infected individuals. Chronic HIV infection (is called asymptomatic HIV infection or clinical latency) includes a long period of clinical latency (～8–10 years) before the development of AIDS, in association with decreasing CD4+T lymphocyte cell counts and increasing levels of HIV viremia. AIDS is the final stage of HIV infection that has severely damaged the immune system.[(3)(4)]

Quantification of HIV-1 viral load is strongly predicts the rate of decrease in CD4+ lymphocyte count and progression to AIDS and death and is more effective to monitoring antiretroviral therapy to reduce the HIV virus a lot of HIV VL(Viral Load) assay have a similar specification using a own MDx(Molecular diagnostics) *AccuPower*® HIV-1 Quantitative RT-PCR Kit allow detection of diverse group M subtype and group O with vacuum-drying, increase a product stability and BIONEER`s own MDx system from nucleic acid extraction to qPCR. Using *ExiStation*™ System and *ExiStation*™ Manager software is the more friendly than other HIV-1 VL assay system.

## 3. FEATURES AND PRINCIPLE OF THE TEST

Real-time PCR involves the selective amplification of a dual target sequence (HIV-1 gag-pol gene and LTR region) while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. PCR Reverse transcriptase from the initial RNA promotes the synthesis of the cDNA. After the synthesis, PCR amplification by DNA Polymerase proceeds. The specificity is provided by a pair of specific primers, along with a hydrolysis probe which is also sequence specific. Monitoring amplified product is conducted by labeling the hydrolysis probe with a matched pair of fluorescent dyes (5'- Fluorescent reporter; 3'- Quencher). Due to fluorescence resonance energy transfer (FRET), an intact probe will not emit light. However, upon cleavage by the 5' – 3' exonuclease activity of the DNA polymerase during PCR, the fluorescent reporter molecule will emit a specific wavelength of light within the visible

spectrum when cleaved after binding to the amplicon[1].

The kit was designed to maximize reproducibility and ease-of-use by vacuum-drying all reagents for PCR including primers, probes, DNA polymerase, dNTPs and salts by using our proprietary stabilization technology to preserve the full activity of the mixed reagents. The primer-probe set was selected from a pool of primer- probe combinations designed by bioinformatics algorithms to achieve maximized amplification efficiency and to match the thermal cycler program with all of our other *AccuPower*® Diagnostic Kits. So that, this product could be run simultaneously with other kits from *AccuPower*® Diagnostic Kit series.

## 4. CONTENTS AND RELATED INSTRUMENTS

### 4.1 Contents of the Kit


**Table 1. Components of *AccuPower*® HIV-1 Quantitative RT-PCR Kit**

| No. | Reagent | Unit | Components | Safety symbol and warning | Quantity | Function |
|---|---|---|---|---|---|---|
| ① | **HIV-1 Premixed qPCR tubes** | **8-well strip X 12 ea (96 tests)** (in aluminum foil bag) | Tris buffer, potassium chloride, magnesium chloride, primer/probe for HIV-1 detection, DL-Dithiothreitol, 0.01% Tween 20, RNA transcriptase, RNase Inhibitor |  | 1 pack | NA amplification |
| ② | **HIV-1 SPC[a] (S1)** (4,000 cp/mL) | **1300 µL / tube (Green 2 mL screw tube)** | Non-infectious virus particle(non-infectious RNA in TYMV) construct containing primer/probe specific region, DEPC-DW, 0.05% Acetylated Bovine serum albumin |  | 1 tube | Calibration |
| | **HIV-1 SPC (S2)** (40,000 cp/mL) | | | | 1 tube | |
| | **HIV-1 SPC (S3)** (400,000 cp/mL) | | | | 1 tube | |
| | **HIV-1 SPC (S4)** (4,000,000 cp/mL) | | | | 1 tube | |
| | **HIV-1 SPC (S5)** (40,000,000 cp/mL) | | | | 1 tube | |
| ③ | **HIV-1 LPC[b]** (4,000 cp/mL) | **1300 µL / tube (Blue 2 mL screw tube)** | Non-infectious virus particle(non-infectious RNA in TYMV) construct containing primer/probe specific region, DEPC-DW, 0.05% Acetylated Bovine serum albumin |  | 3 tubes | Positive Control |
| | **HIV-1 HPC[c]** (400,000 cp/mL) | **1300 µL / tube (Red 2 mL screw tube)** | Non-infectious virus particle(non-infectious RNA in TYMV) construct containing primer/probe specific region, DEPC-DW, 0.05% Acetylated Bovine serum albumin | | 3 tubes | Positive Control |
| ④ | **HIV-1 NTC[d]** | **1300 µL / tube (Clear 2 mL screw tube)** | DEPC-DW, 0.05% Acetylated Bovine serum albumin | | 3 tubes | Non Template Control |
| ⑤ | **SL buffer** | **1300 µL / tube (Clear 2 mL screw tube)** | DEPC-DW, 0.05% Acetylated Bovine serum albumin | | 2 tubes | Sample dilution |
| ⑥ | **Optical sealing film** | - | - | | 1 sheet | Sealing of premix well |
| ⑦ | **Quick Manual** | - | - | | 1 ea | |
| ⑧ | **User Guide** | - | - | | 1 ea | Download via URL |

**a : Standard Positive Control b : Low Positive Control c : High Positive Control d : Non Template Control**

**[Hazard Identification]**

Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

H303 May be Harmful if swallowed.
H315 Causes skin irritation.
P264 Wash hands thoroughly after handling.
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P302+P352 IF ON SKIN : Wash with plenty of water.
P321 Specific treatment (see supplemental first aid instructions on this label).
P332+317 If skin irritation occurs: Get medical Help.
P362+364 Take off contaminated clothing and wash it before reuse.

### 4.2 Related Instruments

This kit is optimized for use with BIONEER's *ExiStation*™ Universal Molecular Diagnostic System (A-2200, A-2200-N, A-2400, A-2410). For detailed operating instructions of each device, please refer to the instrument *User's Guide*.

## 5. STORAGE CONDITION AND SHELF LIFE

The *AccuPower*® HIV-1 Quantitative RT-PCR Kit should be stored at -25 ~ -15℃ away from UV/sunlight. The kit is guaranteed stable until the expiration date (12 months) printed on the label. Repeated thawing and freezing of HIV-1 premixed qPCR tube, the SPCs (HIV-1 SPC (S1) - (S5)) and PCs (HPC/LPC) should be avoided, as this may reduce assay performance. If intermittent use of the kit and component (HIV-1 premixed qPCR tube, the SPCs and PCs) is expected, HIV-1 premixed qPCR tube are stable for up to 10 freeze/thaw cycles and SPCs (HIV-1 SPC (S1) - (S5)) / PCs (HPC/LPC) are stable for up to 3 freeze/thaw cycles.

## 6. REQUIRED MATERIALS AND EQUIPMENT

*ExiStation* system includes *ExiPrep*™ Dx instrument for automated nucleic acid extraction and the *Exicycler*™ for real-time PCR.

| System | Instrument | Reagent (Extraction) |
|---|---|---|
| ***ExiStation*™ (A-2200)** | -*ExiPrep*™16 Dx (Cat. No. A-5050)<br>-*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060) | - *ExiPrep*™ Dx Viral RNA Kit (K-4473)<br>- *ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471)<br>- Sample Loading Tube_RNA IPC (Cat. No. KA-3011) |
| | *Existation*™ manager software (Version 1.02.XX) | |
| ***ExiStation*™ (A-2200-N)** | - *ExiPrep*™16 Dx (Cat. No. A-5050)<br>- *Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1) | - *ExiPrep*™ Dx Viral RNA Kit (K-4473)<br>- *ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471)<br>- Sample Loading Tube_RNA IPC (Cat. No. KA-3011) |
| | *Existation*™ manager software (Version 4.02.XX) | |
| ***ExiStation*™ 48 (A-2400)**<br>***ExiStation*™ 48A (A-2410)** | - *ExiPrep*™48 Dx (Cat. No. A-5150)<br>- *Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1)<br>- *ExiLT* (Cat.No. A-7100) | - *ExiPrep*™48 Viral DNA/RNA Kit (K-4571)<br>- *ExiPrep*™48 Viral RNA Kit (K-4573)<br>- *Exiprep*™48 Sample Loading Tube_RNA IPC (KA-4502) |
| | *Exiprep*™ 48 software (Version 1.0.X.X) | |
| **Etc** | - *ExiSpin*™ (Cat.No. A-7040) | N/A |
| **General lab- equipment and disposables** | • Disposable powder-free gloves<br>• Pipette set appropriate volume (1,000 µL, 200 µL, 20 µL pipette)<br>• Sterilized pipette tips with filters (1,000 µL, 200 µL, 20 µL tips with filters)<br>• 1.5 mL or 15 mL conical tubes | |

## 7. GENERAL PRECAUTIONS

- Real-Time PCR with this kit should be performed using *Exicycler*™ 96 Real-Time Quantitative thermal block.
- Please read this User's Guide before use.
- All patient`s specimens should be handled as infectious material.
- Always wear gloves, laboratory coat and a mask when handling specimen or agents.
- Change gloves after contact with potential contaminations, e.g. specimens, eluents, etc.
- Wash hands thoroughly after handling specimen and reagents and taking off the gloves.
- Do not pipette by mouth.
- Do not eat, drink or smoke in dedicated working area.
- DO NOT re-use opened reagents and do not mix reagents from different production lots.
- DO NOT change the protocol as described in this User's Guide.
- Always use sterile, disposable filtered-pipette tips.
- Clinical samples and their derivatives should be stored in a separate location/ freezer from where the rest of the kit components are stored.
- DO NOT freeze whole blood or any samples stored in primary tube.
- The SPC 1~5, LPC, HPC, and SL buffer should be thawed 15~30°C. After thawing the SPC 1~5, LPC, HPC, and SL buffer, briefly vortex for 3 seconds and spin-down.

After thawing the SPC, LPC, HPC, and SL buffer, the test should be performed immediately.

- Briefly vortex and spin-down all kit components after thawing to ensure optimum results.
- All SPC or PCs should be added in a physically separate location from where the premix is reconstituted.
- Take caution, when using a scissor or cutter.
- Clean and disinfect spilled specimens and/or dedicated working area with 0.5% sodium hypochlorite in distilled or deionized water (1:10 dilution of liquid household bleach) and should be thoroughly rinsed with 70% ethanol or distilled water.
- DISCARD A WASTE (liquid, plastic ware, or biological waste) according to local safety regulation or internal laboratory procedures.

### 7.1 Product's Limitation

- The operator should take care of cross contamination.
- Mutations, deletions, and insertions in the target region may cause false positive / negative results.
- The product has been validated only for use with $K_2$ EDTA plasma. Testing of other sample types may lead to inaccurate results.
- Negative test result does not preclude HIV-1 infection. Results from the product should be interpreted in conjunction with clinical presentation and other laboratory markers.
- Before switching from one technology to the next, recommends that users perform method correlation studies in their laboratory to qualify technology differences.
- Reliable results depend on adequate sample collection, transport, storage, and processing.
- Quantitation of HIV-1 RNA depends on the number of virus particles present in a sample and may be affected by sample collection method, patient factors (i.e., age, presence of symptoms), and stage of infection.
- Sample that yields an INVALID result twice may contain the inhibitors, retesting is not recommended. A new sample must be collected if two consecutive INVALID results were obtained from the same specimen.

# 8. PROTOCOL

## 8.1 Laboratory Equipment and Environment

We recommend that several precautionary measures be taken for the user and the laboratory safety and the prevention of laboratory environmental contamination.

When handling clinical samples, all related work (de-capping, pipetting, and capping of clinical samples and containers) **should be conducted within a negative pressure biosafety cabinet (class II or III).** A negative pressure biosafety cabinet sends air from the laboratory space to outside. In other words, air flows inwards. This airflow prevents dangerous substances from contaminating the laboratory environment.

When opening sterilized containers such as Buffer Cartridges (*ExiPrep*™ Dx prep kit series), the work should be conducted in a positive pressure environment to prevent environmental contaminants from entering and fouling the sterile supplies. A Cleanbench is a workspace were filtered air flows outwards, thus keeping a clean environment within the workspace.


**Fig. 1** Biosafety Cabinet (BSC) & Cleanbench

## 8.2 Specimen

The *AccuPower*® HIV-1 Quantitative RT-PCR Kit has been validated for use as a quantitative assay with only human EDTA plasma.

### 8.2.1 Specimen Collection and Pretreatment

Whole blood should be collected in sterile tubes using $K_2$ EDTA (lavender top) as the anticoagulant. To obtain the 400 µL reaction volume, the minimum volume of plasma for collection tubes is up to 500 µL.

The $K_2$ EDTA (lavender top) tube in which the blood was collected is mixed for 5 minutes in the roller mixer, then centrifuged at 1,300 g for 10 minutes [6].

### 8.2.2 Specimen Transport

All samples should be transported in a shatterproof transport container to prevent potential infection from sample leakage. Samples should be transported according to local/national guidelines regarding biohazard transportation. Whole blood collected in EDTA tubes should be stored and/or transported within 24 hours at 2°C to 30°C.

### 8.2.3 Specimen Storage

The isolated human EDTA-plasma can be stored up to 7 days between 2°C and 8°C or up to 4 weeks between -80°C and -15°C. Plasma samples are stable for up to 3 freeze/thaw cycles when stored frozen between -25°C and -15°C.

### 8.2.4 Interfering Substances

Clinical samples may contain a variety of PCR inhibitors. For efficient PCR, such inhibitors must be removed during the RNA extraction and purification process.

For the optimal PCR results, the interference materials in the specimens would be eliminated during the RNA extraction process using the *ExiStation*™ Universal Molecular Diagnostic System.

### 8.2.5 Exclusion Criteria for Specimen[7]

The specimens with the following conditions must be excluded.

- Specimens visually hemolyzed or containing lipid.
- Specimens coagulated despite anticoagulant containers.
- Specimens with inappropriate storage conditions.

## 8.3 Work Flow

The *AccuPower*® HIV-1 Quantitative RT-PCR Kit is designed for use with *ExiStation*™ Universal Molecular Diagnostic System.

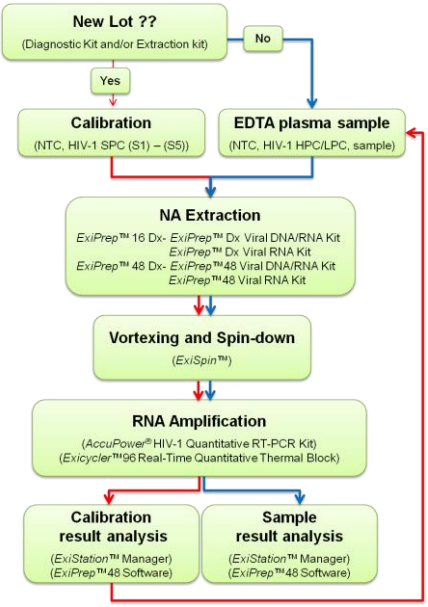



**Fig. 2** Work flow

Nucleic acid extraction and PCR should be conducted according to the protocol described in this User's Guide when using the kit with *ExiStation*™. The PCR can be performed without additional steps for preparing PCR mixture when *ExiStation*™ Universal Molecular Diagnostic System is used. After completing the PCR process, the data can be automatically analyzed through *ExiStation*™ Manager software. For further instructions, please refer to this User's Guide (Section 8.4 and 8.5 of the procedure).

## 8.4 Experimental Procedure I (*ExiStation*™ system)

### Part 1. Assigning test using *ExiStation*™ Manager software

*** The *ExiStation*™ Universal Molecular Diagnostic System utilizes automated nucleic acid extraction on the *ExiPrep*™16 Dx instrument with *ExiPrep*™ Viral DNA/RNA Kit (K-4471) or *ExiPrep*™ Viral RNA Kit (K-4473). For further information on the extraction, please refer to the User's Guide of K-4471 or K-4473.**

1) Turn on the computer, which is preinstalled with *ExiStation*™ Manager software.

2) Execute the *ExiStation*™ Manager software by clicking the icon located on the desktop.


**Fig. 3** ***ExiStation*™ Manager Software icon**

3) Turn on the *ExiPrep*™16 Dx (A-5050) by pressing the main power button located at the front of the instrument. Press the "STARTING" button displayed on the LCD to initiate instrument startup.


**Fig. 4 Starting button and main power button of *ExiPrep*™16 Dx**

4) Press the "MISC SET" button on the LCD screen (or the "Load" button on the software).


**Fig. 5 LCD screen of *ExiPrep*™16 Dx and Load button of *ExiStation*™ Manager Software**

5) Apply the filter paper onto the Contamination Shield. Attach the prepared Contamination Shield, then the Tip Protector in the instrument. Press the "Misc Set" button again.

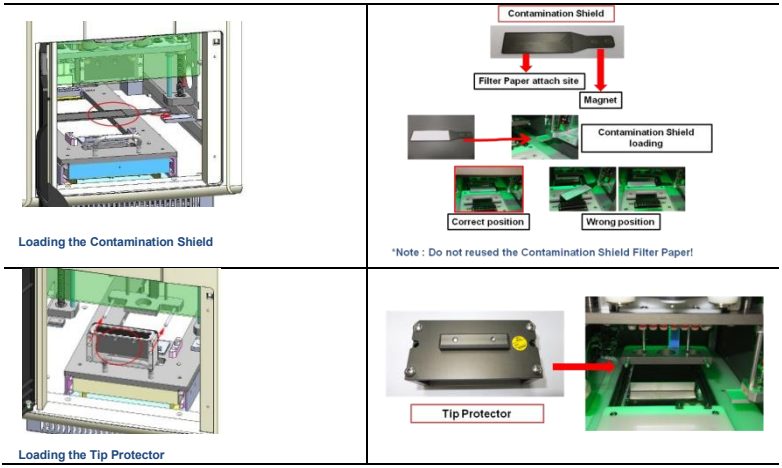



**Fig. 6** Location and mounting method of the contamination shield and tip protector

6) Close the instrument door and press the “UV lamp” button on the LCD screen.


**Fig. 7** LCD screen of *ExiPrep*™16 Dx

7) *ExiStation*™ Manager software has 6 distinct parts.

**Prep** - controls nucleic acid extraction (*ExiPrep*™16 Dx instrument),
**Assign PCR** - transfers sample information from “Prep” to “PCR” (*Exicycler*™ 96) and assigns for PCR run
**PCR** – displays real-time amplification conditions (*Exicycler*™ 96)
**Result** - presents the results, the experiment information, and the sample information after the PCR process has been completed
**Configuration** - software setup information (accessible only by the manufacturer)
**Version** - displays software version

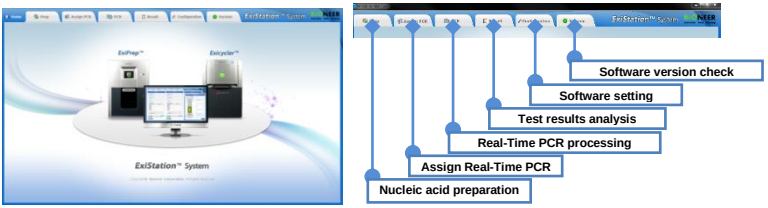



**Fig. 8** Tab function of *ExiStation*™ Manager software

8) Click the "**Prep"** tab on the upper left of the main screen to initiate the nucleic acid extraction process.

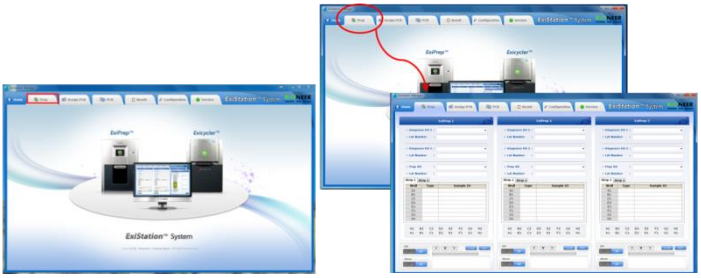


**Fig. 9** Prep control panel consist of 5 panels

Prep control panel consists of 5 panels.

**Instruments status panel** – displays the status of *ExiPrep*™16 Dx
**Kit selection panel** – allows selection/input of the diagnostic kit, prep kit, and lot information (optional: barcode scanning system)
**Sample and control information panel** – allows input of control (NTC, PC, SPC) and sample information (optional: barcode scanning system)
**Well information panel** – displays the well information with different colors
***ExiPrep*™16 Dx control panel** – controls *ExiPrep*™ 16 Dx including UV controller, Store controller, Running controller, and MISC set controller

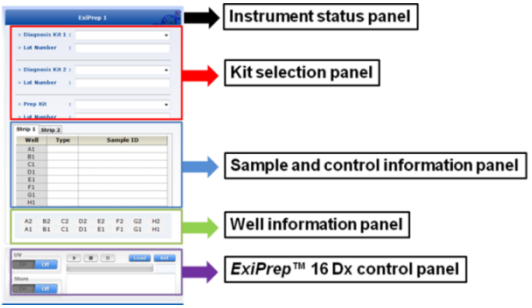



**Fig. 10** Prep control panel of *ExiStation*™ Manager software

9) Click the pull-down arrow for “Diagnosis Kit 1”. A popup will appear and select “HIV-1111” from the pull-down menus.

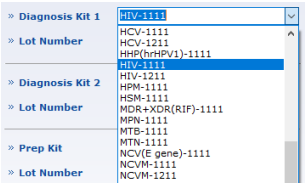



**Fig. 11** Selection of Diagnostic kit

10) After selecting the “Diagnosis Kit”, a popup will appear. Inspect the Buffer Cartridge and mark the used well by clicking on the corresponding location to exclude the used well from the sample assignment. Select the “OK” to finish.


**Fig. 12** ‘Prep’ Pop-up window of *ExiStation*™ Manager software

11) Click the pull-down arrow for “Prep Kit”. A popup of the appropriate “Prep Kit” for the selected diagnostic kit will automatically appear. Select “Prep Kit” from the pull-down menus.

12) Enter lot number of the diagnostic kit and the prep kit. The program will automatically allocate the NTC and SPC (or PCs) wells.
The lot of diagnostic kit and/or extraction kit is new, the program automatically assigns the NTC and SPC from 1 to 5. When the same lot combination of the diagnostic kit and the extraction kit is used to the previous assay, the standard curve is automatically saved, and only 1 LPC (Low Positive Control) and 1 HPC (High Positive Control) are assigned as a positive control.

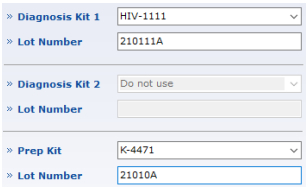



**Fig. 13** Entering lot number

13) Click the “Sample ID” column and enter sample information either by typing in manually or using a barcode reader (optional).

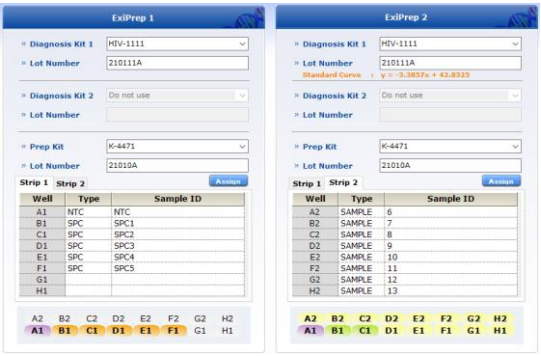



**Fig. 14** Enter Sample ID (First assay/Repeated assay)

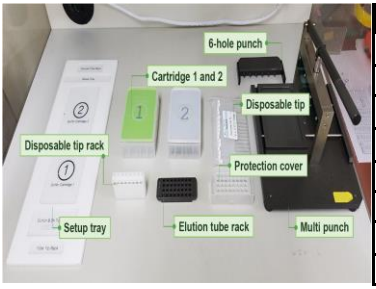
## Part 2. Nucleic acid extraction by *ExiPrep*™ 16 Dx

1) BIONEER recommends using the BSC (Class Ⅱ) and clean bench for *ExiStation*™ system operation.

2) Clean the surface (preferably a Cleanbench-1) where work will be performed.

> ⚠ **Clean the surface with 0.5% sodium hypochlorite in distilled or deionized water and rinse with distilled water or 70% EtOH, before and after use to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.**
>
> ⚠ **Turn off the UV lamp when using the BSC.**

3) Prepare of nucleic acid extraction kit in PCR station 1.




| No. | Components | Component of |
|---|---|---|
| 1 | Setup tray | *ExiPrep*™ 16 Dx |
| 2 | Cartridge 1 and 2 | Extraction Kit |
| 3 | Disposable tip rack | *ExiPrep*™ 16 Dx |
| 4 | Elution tube rack | *ExiPrep*™ 16 Dx |
| 5 | Disposable tip | Extraction Kit |
| 6 | Protection cover | Extraction Kit |
| 7 | 6-hole punch | *ExiPrep*™ 16 Dx |
| 8 | Multi punch | Optional |

**Fig. 15** List of necessary components for nucleic acid extraction

4) Remove the shrink-wrap enclosing the both Buffer Cartridges ① and ② then remove the lids.

> ⚠ **Inspect the wells of the Buffer Cartridges and make sure all liquids are at the well's bottom.**

**Fig. 16** Remove the lids

5) Punch the film with the Hole Puncher according to the layout mapped on the software.

> ⚠ **Since improper punching of the film may cause malfunction of the instrument. Push in Hole Puncher firmly to ensure that Buffer Cartridge is adequately punched.**

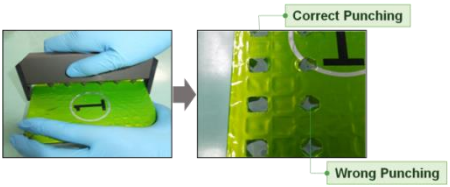



**Fig. 17** Punch the film with the Hole Puncher

6) Cover Buffer Cartridges ① and ② with the lids after film punching is complete.

7) Place Buffer Cartridges on the setup tray.

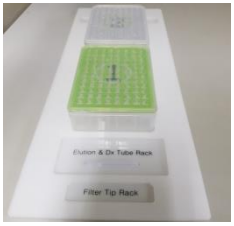



**Fig. 18** Install buffer cartridge on the set-up tray

8) Take the necessary number of strips of the Diagnostic Kit Tubes from the freezer. Remove the foil covering the tubes. Insert appropriate numbers of Diagnostic Kit Tubes into the Elution Tube Rack. It is recommended to mark each strip of the diagnostic tubes with the corresponding column number.

> ⚠ **Ensure that the diagnostic tubes are marked so they can be identified later.**
> ⚠ **At the bottom of the Elution Tube Rack, there is a groove fitted to the *ExiPrep*™16 Dx instrument. When viewed from above, place the groove side downwards and insert the premix tubes into two upper rows.**

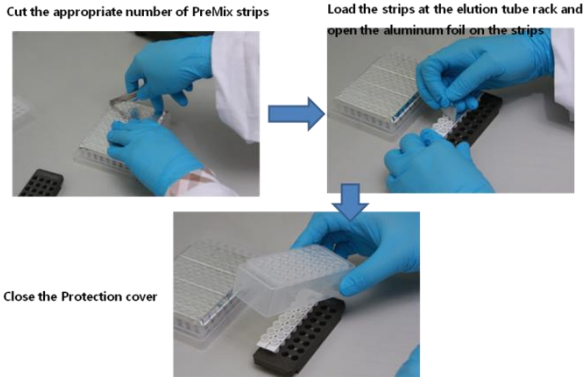



**Fig. 19** Inserting the *AccuPower*® Diagnostic Kit tubes into Elution Tube Rack

9) Fasten the Protection Cover onto the Elution Tube Rack. Place Elution Tube Rack (containing Diagnostic Kit) on the setup tray.

10) Load the appropriate number of disposable filter tips at the disposable tip rack.

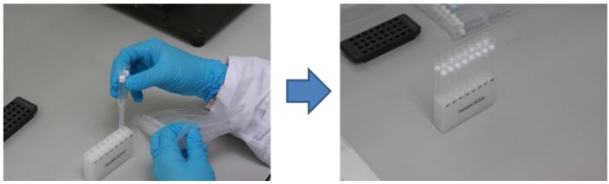


**Fig. 20** Load the disposable filter tips at the disposable tip rack

11) Place the disposable tip rack on the setup tray.

12) Place the waste tray on the setup tray.

**Fig. 21** Install Disposable filter tip and setup tray to the setup tray

13) Open the door of the *ExiPrep*™16 Dx (A-5050) and pull the Base Plate out completely. Starting from the Buffer Cartridges, place each component one-by-one into the Base Plate as described below.

14) Place the Buffer Cartridge ② on the heating block of the base plate.

> ⚠ **If Buffer Cartridge ② is not properly placed on the heating block, it results in experiment failure or an instrument malfunction.**

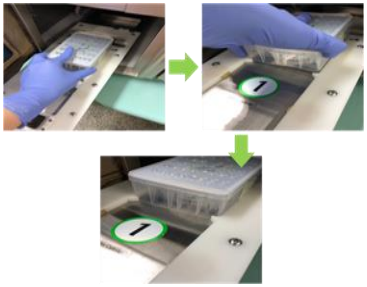


**Fig. 22** Place the Buffer Cartridge ②

15) Place the Buffer Cartridge ① on the base plate.

> ⚠ **Place the Buffer Cartridge ① slightly tilting the cartridge to the left side of the base plate and firmly press the cartridge's right-hand side.**

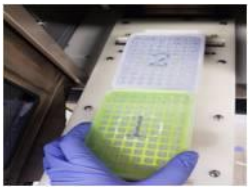


**Fig. 23** Place the Buffer Cartridge ①

16) Place the Elution Tube Rack and Disposable Tip Rack on the base plate.

> ⚠ **Check the Protection Cover is adequately secured on the Elution Tube**
> ⚠ **Ensure the tips, holes, and tubes are in alignment.**

**Fig. 24** Inserting the Disposable Tips into the Disposable Tip Rack

17) Place the Waste tray in between the Sample Tube Rack and the Buffer Cartridge ②.

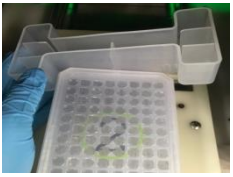


**Fig. 25** Loading the Waste tray

18) Slide the Base Plate in and close the door of the *ExiPrep*™ 16 Dx. Keep the door closed until the Sample Loading Tube is ready.

> ⚠ **When slide the base plate in, gently push the base plate not to spill the samples and reagents.**

19) Prepare clinical samples, sample loading tubes, and controls in BSC. Clean the negative pressure BSC on which the nucleic acid extraction preparation will be performed.

> ⚠ **Clean the surface with 0.5% sodium hypochlorite in distilled or deionized water and rinse with distilled water or 70% EtOH, before and after use to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.**
>
> ⚠ **Turn off the UV lamp when using the BSC.**

**Fig. 26** Necessary components preparing for sample loading

20) Please take out the RNA IPC Sample Loading Tubes from the packaging, mark it with a sample name and insert them into the rack.

> ⚠ **Before using a Sample Loading Tube, the bottom of the Sample Loading Tube MUST BE check for Yellow color (dried IPC for RNA)**

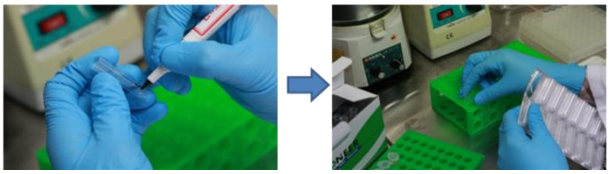


**Fig. 27** Preparing Sample Loading Tube

21) Take the original clinical sample containers and controls (NTC and SPC) and pipette into the RNA IPC Sample Loading Tubes by following steps 22) to 25).

22) Add 400 µL of NTC into a tube assigned as NTC (supplied with the *AccuPower*® Diagnostic Kit).

23) Additionally, add 400 µL SPC 1~5 into the appropriate SPC wells (supplied with the *AccuPower*® Diagnostic Kit).

> ⚠ **<u>If you have the pre-date of the same lots of Diagnostic kit and Extraction kit</u>, you may skip SPC calibration. By the Standard, information saves automatically, in this case, NTC, LPC, and HPC role as control.**
> **<u>When the assay is repeated with the same lot of Diagnostic kit and Extraction kit</u>**
>
> **NTC**: Load SL buffer **400 µL in NTC tube.**
> **LPC**: Load LPC **400 µL** (**blue cap tube**, the component of *AccuPower*® Diagnostic Kit)
> **HPC**: Load HPC **400 µL** (**red cap tube**, the component of *AccuPower*® Diagnostic Kit)

24) Move the filled Sample Tube into the Sample Tube Rack.

> ⚠ **Insert the Sample Tubes vertically to prevent spilling.**

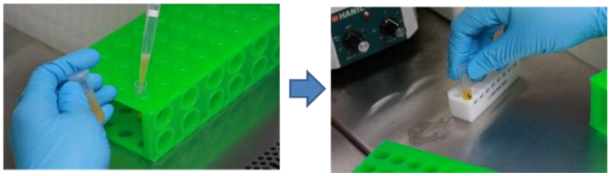


**Fig. 28** Load clinical sample to Sample Loading Tube

25) Uncap clinical sample container and pipette 400 µL sample into RNA IPC Sample Loading Tube. Move the RNA IPC Sample Loading Tube into Sample Tube Rack when it is filled with sample.

26) Repeat the sample loading steps individually until all samples are loaded.

> ⚠ **In case of any contamination of tips or gloves are suspected, immediately change gloves and tips to prevent the sample contamination.**

27) Remove the waste tray on the base plate.

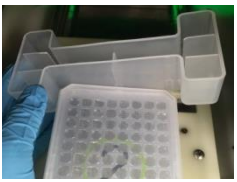


**Fig. 29** Remove the waste tray

28) Load the sample tube rack on the *ExiPrep*™ 16 Dx base plate.

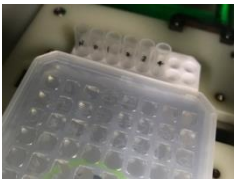


**Fig. 30** Install of Sample Tube Rack

29) Place the Waste tray in between the Sample Tube Rack and the Buffer Cartridge ②.

> ⚠ **Be careful not to tip over the Sample Tube Rack.**

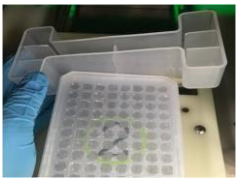


**Fig. 31** Re-load Waste Tray

30) All materials are loaded.

31) Remove the lids from Buffer Cartridges.

> ⚠ **Ensure the lids of the Buffer Cartridges are removed and all components are in the correct position.**

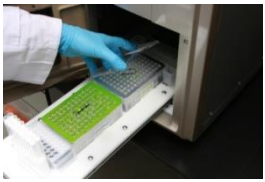


**Fig. 32** Remove the lids

32) Check whether all accessories are loaded properly.

| ⚠ | **Ensure the tips, holes, and tubes are all in alignment.** |
|---|---|

33) Push the base plate carefully and close the door.

| ⚠ | **Gently push the base plate in to prevent any sample or reagent spilling.** |
|---|---|

## Part 3. Running *ExiPrep*™ 16 Dx and *Exicycler*™ 96 using *ExiStation*™ manager software

*** Please refer to the Equipment User's Guide for basic instructions on using *Exicycler*™ 96 and *ExiStation*™ Manager software.**

1) Click the 'RUN (▶)' button of the ***ExiStation***™ Manager Software. Double-check whether all accessories are adequately loaded according to the 'Check ***ExiPrep*** Setting' list and check the boxes. Click 'OK' button to initiate the prep process.

> ⚠ **The nucleic acid extraction process takes 80~100 minutes according to the type of nucleic acid.**
> ⚠ **Suppose any error messages appear during the extraction process. Contact local BIONEER`s distributor or headquarter for technical assistance.**

**Fig. 33** Click the 'RUN' button on *ExiStation*™ Manager software

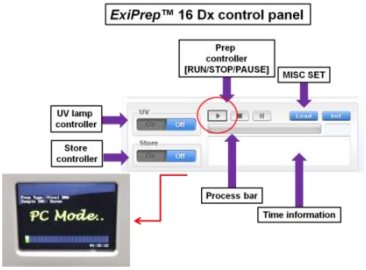



**Fig. 34** *Exiprep*™ 16 Dx control panel

2) When the nucleic acid extraction process is finished, the cooling block is automatically turned off. Open the door of *ExiPrep*™16 Dx (A-5050) when the nucleic acid extraction process is complete, and remove the Elution Tube Rack.

> ⚠ **When sliding the base plate out, gently pull the base plate not to spill the waste.**

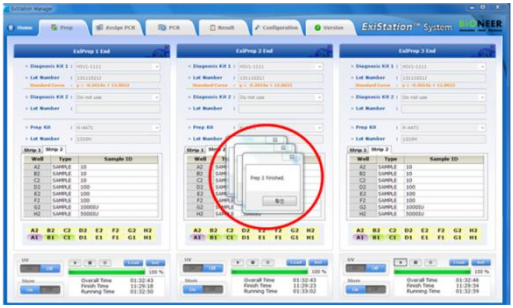


**Fig. 35 Pop-up message for extraction finished**

3) Move the elution tube rack to PCR station 2.

**Fig. 36 PCR preparation**

4) Please remove Protection Cover according to Protection Cover Separation Tool utility method.

> ⚠ **When nucleic acid extraction is finished, the next step should be progressed within 10 minutes. If not, this may lead to an inaccurate result.**

① Take out Elution Tube Rack from *ExiPrep*™16 Dx and place it on top of Protection Cover Separation Tool.
Note: When placing the Elution Tube Rack on Protection Cover Separation Tool, the lever must be facing the left-hand side.

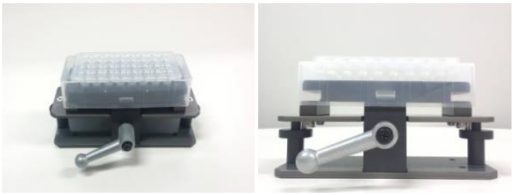


**Fig. 37** Picture of Elution Tube Rack on top of Protection Cover Separation Tool

② Firmly hold down Protection Cover and Separation Tool with one hand. Rotate the lever in a clockwise 180° with the other hand.

Note: Rotate the lever until Elution Tube Rack is firmly fixed to Protection Cover Separation Tool.

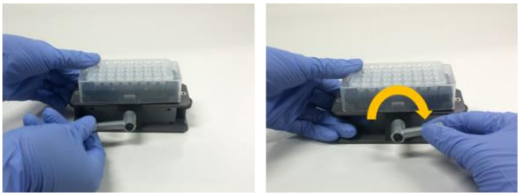


**Fig. 38** Picture of lever rotation for fixing Elution Tube Rack to Protection Cover Separation Tool

③ Press both sides down of the Separation Tool shown below. This action will push Protection Cover upwards so that Elution Tube Rack can be removed with ease.
Tip: Hold down Protection Cover with one hand. Then press down each side of the Separation Tool consecutively to prevent any liquid from splashing.

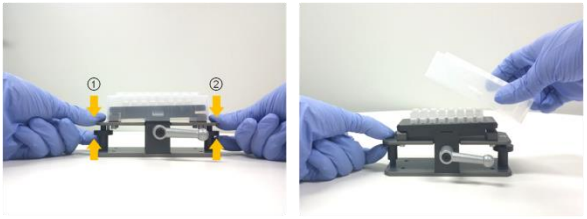



**Fig. 39** Picture of pressing down each side of Separation Tool and removing Protection Cover from Separation Tool

5) Seal PCR Tube using Optical sealing film and then proceed to the next step. For more information on the Sealing process, refer to step 6).

6) Seal the Diagnostic Tubes with the adhesive Optical Sealing Film.

> ⚠ **In order to avoid contaminations and invalid results, seal all the tubes thoroughly.**
> ⚠ **Store the sealed diagnostic tubes at 4°C until use (if the prep reaction is divided into 2 steps, store it until 2nd prep finishes).**

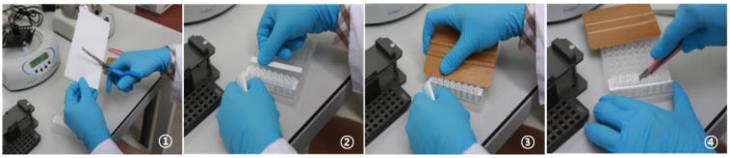


**Fig. 40** Seal PCR premix strip

7) Right before the PCR reaction, completely mix the tube contents using ***ExiSpin***™ (A-7040). (*ExiSpin*™ parameters: 2500rpm for 1 sec., Hard vortex for 20 sec./ 20 cycles)

> ⚠ **BIONEER's PCR premix contains vacuum-dried PCR reagents. Insufficient mixing could result in invalid PCR results, so mix until the premix is thoroughly dissolved.**
> ⚠ **Ensure to mark each diagnostic kit to prevent mix up.**
> ⚠ **When nucleic acid extraction is finished, the next step should be progressed within 10 minutes. If not, this may lead to an inaccurate result.**

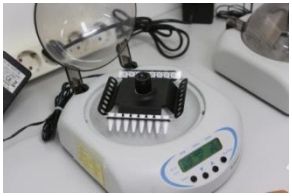


**Fig. 41** Mix the PCR Premix Strip using *ExiSpin*™

> ⚠ **DO NOT manipulate *ExiSpin*™ protocol, arbitrarily**
> ⚠ **HAVE TO adjust the balance**

8) While *ExiSpin*™ is operating, turn the *Exicycler*™ 96 Power Switch on located at the rear of the instrument. The LED status light in front of the instrument should turn Blue. Press the Power Switch for 3 seconds. A brief self-test sequence will initiate. When the self-test is completed, the LED will blink GREEN with a short beep.


**Fig. 42** Operation button (door button, power button and status LED) of ***Exicycler*™ 96**

9) Click the 'Assign PCR' tab on the main screen of the *ExiStation*™ Manager program.
The 'Assign PCR tab' consists of six tabs.

| | |
|---|---|
| **Assign** | Assign the Prep WorkList on 96 well plates, marked the strip number. |
| **Current Step** | It indicates the progress of nucleic acid extraction in *ExiPrep*™ 16 Dx.<br>Prep: middle of nucleic acid extracting / Prep End: Finish the nucleic acid extraction |
| **Diagnosis Kit** | In Prep WorkList, displayed the diagnostic kit that has been extracted. A selected diagnostic kit can operate PCR with other diagnostic kits at the same time. |
| **Prep Kit** | It indicates the used extraction kit in prep WorkList. |
| **Start Time** | It indicates the start time of nucleic acid extraction. |
| **Finish Time** | It indicates the finish time of nucleic acid extraction. |

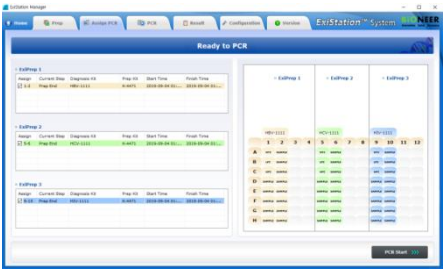



**Fig. 43** PCR Random Access

10) Click the 'Assign PCR' tab and check the box of each 'Prep Work List' to assign PCR position. PCR position correspond to *ExiPrep*™ 16 Dx #1~3 in order.

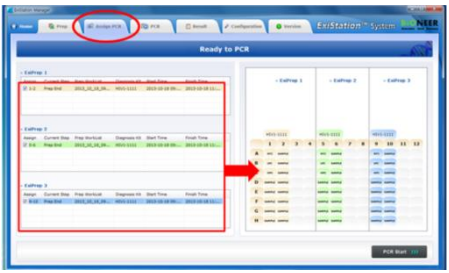



**Fig. 44 'Assign PCR' tab - PCR Start**

11) Push the Door Switch for 2 seconds to slide the 96-well thermal block out. Insert the reaction tubes in their locations. When sample loading is completed, push the Door Switch for 2 seconds to close the door.

| | |
|---|---|
| ⚠ | **Ensure the sample loading configuration is agreement with the assigned well position.** |
| ⚠ | **If you are running less than 6 strips for a PCR run, please insert a dummy strip at the opposite end (column 12) to balance out the pressing force of the hot lid in *Exicycler*™ 96.** |

12) Place the mixed premix tubes into the assigned well position of *Exicycler*™ 96 when cycling is completed. For detailed operation instructions of *Exicycler*™ 96 and *ExiStation*™ Manager software, see the relevant ***User Guide.***

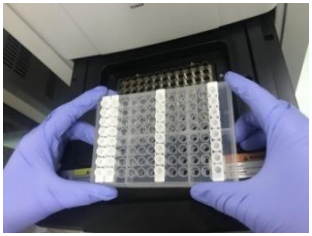


**Fig. 45 Way to PCR Premix Strip setup of *Exicycler*™ 96**

13) Select the 'Assign PCR' tab and confirm the assigned 'Prep Work List.' After the 'Prep' process, 'Current Step' will be presented as 'Prep End,' and the upper-status bar will be changed to 'Ready to PCR.' Initiate PCR run by clicking the activated 'PCR Start' button at the window's bottom right-hand side.

A popup window will appear, prompting the user to enter a Work List Name. Click 'OK' after entering a name to generate a Work List for Real-Time PCR.

⚠ **Default Work List file path is 'C: > *ExiStation*_Data > user > GUEST > WorkList'.**

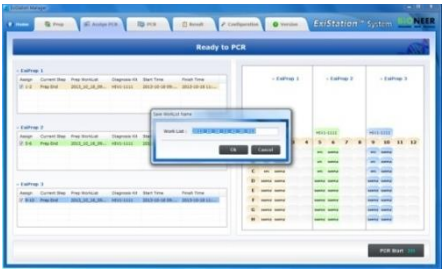



**Fig. 46** Pop-up window of "Data name"

14) After entering the Work List Name, the 'PCR' tab will be activated, and the ***Exicycler***™ 96 will automatically initiate the PCR run.

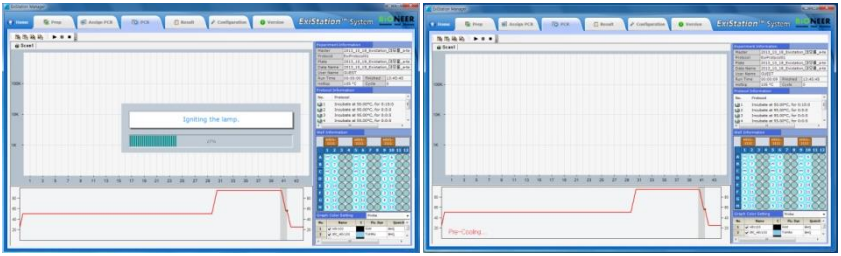



**Fig. 47** PCR Running screen

15) Remove all consumables and components, starting with the Buffer Cartridges and various racks from the instrument, and discard all liquids and consumables in their appropriate containers.

⚠ **If un-used wells are present in the Buffer Cartridges, take a lint-free cloth or 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the lids on the Buffer Cartridges and keep them in a Cleanbench-1 for later use.**

⚠ **Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.**

16) Press the 'Misc Set' button, remove Tip Protector and Contamination Shield, then press the 'Misc Set' button again

17) Push the Base Plate in, shut the instrument door, and initiate UV sterilization by clicking "UV ON" on the control panel.

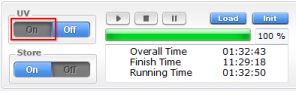



**Fig. 48** ***ExiPrep***™**16 Dx control panel – UV**

18) After the PCR run is finished, select the 'Result' tab to check each samples result.

| | |
|---|---|
| ⚠ | **Click the 'Analysis' button to open the dedicated analysis popup, which presents detailed results, including a fluorescence graph.** |
| ⚠ | **DO NOT peel off an optical sealing firm from Diagnostic Kit. Discard them according to local safety regulations or internal laboratory procedures.** |


**Fig. 49 Result analysis using *ExiStation*™ Manager software**

19) The result data files are saved in 'C: > *ExiStation*_Data > user > GUEST > WorkList > relevant data file name' folder.

## 8.5 Experimental procedure Ⅱ (*ExiStation*™ 48, *ExiStation*™ 48A)

### Part 1. Assigning test using *ExiPrep*™48 software

*** Please refer to the user guide of *ExiPrep*™48 Viral DNA/RNA Kit, *ExiPrep*™48 Dx, or *ExiLT* for basic workflow.**

1) Turn the *ExiPrep*™ 48 Dx switch on the back of the instrument, and press the POWER button on the front of the instrument for over 1 second.

2) As it starts to initialize, the LCD screen will automatically appear.

3) When the initialization of the instrument is completed, the main screen appears on the LCD screen. If initialization is NOT successfully completed, contact BIONEER or related agencies.


**Fig. 50 Main screen of *ExiPrep*™ 48 Dx**

4) Main screen consists of 5 icons.

**Prep** – Set-up and control nucleic acid extraction(*ExiLT*™, *ExiPrep*™48 Dx)
**LT** - Automatic de-capping and liquid transfer system
**Assign** – Extracted information can be displayed
**PCR** – Monitoring extraction of Real-Time PCR (*Exicycler*™ 96)
**Result** – Show the results after executing PCR

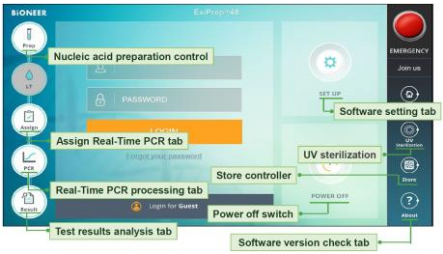



**Fig. 51 Icons function of *ExiPrep*™48 software**

5) Log in with the registered ID. When logging in as a guest, it is usually saved data in the folder specified. Specify a folder efficient resulting data management.

6) Before the Prep, separate the Contamination Shield from *ExiPrep*™48 Dx. Clean with 70% EtOH, attach the Contamination Shield filter paper, and install the prepared Contamination Shield.

**Fig. 52** Decontamination step-1 ; Separation and installation of the Contamination Shield

7) Close the door of the instrument. Click the UV sterilization icon. Select the'15 Minutes' button (Turn on the UV sterilization). After 15 minutes, UV sterilization turns off automatically.

**Fig. 53** Decontamination step-2 ; UV sterilization

8) Touch the Prep icon on the main screen, as shown in figure 54. Enter Prep mode for nucleic acid extraction by touching the ExiStation.


**Fig. 54** Initial screen of Prep tab

9) Touch the pull-down arrow of Diagnosis Kit 1 show a list of available diagnosis kits. Press the HIV-1111.


**Fig. 55** Entering Diagnosis Kit information

10) As pop-up "Select Lane & Well" message, select the well to use. Later check the already used well of Buffer cartridge ①. Click the used well, appear "X" sign upper that well. Finally, click the "OK" button. When there is no used wells to select, straight proceed to next step by clicking the "OK" button.

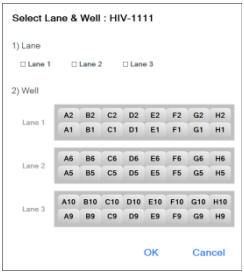



**Fig. 56** Screen of 'Select Lane & Well'

11) Enter Lot number of the diagnosis kit.

12) Touch the pull-down arrow of Prep Kit. Show the prep kit to use. Select the prep kit to use, then enter Lot information of Prep Kit.


**Fig. 57** Entering Kit information

13) As pop-up “Sample Type” message, select the sample to use.

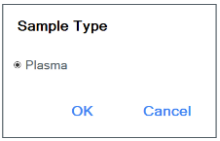



**Fig. 58** Screen of ‘Sample Type’

14) If either Lot number for Diagnosis Kit or/and Prep Kit is new, Notification window required Standard Calibration will be displayed.

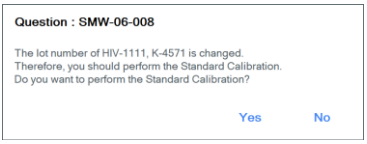



**Fig. 59** Pop-up message for Standard Calibration process

15) The positions of NTC and SPC are automatically displayed in the Buffer Cartridge's remaining wells, and the default setting is set to 1 each of NTC and SPC 1-5. If the experiment is repeated with a combination of extraction/diagnosis kits of the same lot, since the automatically saved standard curve is used, LPC (Low Positive Control) and HPC (High Positive Control) is set for 1 well instead of SPC 1-5.

16) Complete generate of Standard curve normally, proceed the next experiment using clinical samples. If the experiment works, several information appears; standard curve, NTC/LPC/HPC’s right position. Then click the ‘Sample ID’, input the clinical sample’s information (Optional-using barcode reader).


**Fig. 60** Entering sample information

## Part 2. Nucleic acid extraction by *ExiPrep*™ 48 Dx

1) It is recommended that handling clinical samples and all related works should be conducted within a negative pressure BSC (Class II) for user safety and prevention of contamination.

2) Clean the BSC and check all necessary components for extraction and sample before nucleic acid extraction. Prepare extraction components within a Cleanbench-1. Recommend to perform at separated place referring to 8.1.

> ⚠ **Clean the surface with 0.5% sodium hypochlorite and 70% ethanol or D.I water before and after order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.**
> ⚠ **It must be turn off the UV lamp while using the BSC.**

3) Check that all necessary components are present before proceeding and perform the operation within Cleanbench-1.

**Table 2.** List of necessary components for nucleic acid extraction

| Prep tools | Consumables |
|---|---|
| ■ Setup Tray<br>■ Hole Punch<br>■ Sample Tube Rack<br>■ Elution Tube Rack<br>■ Clamp | ■ Buffer Cartridges ① and ②<br>■ Sample Loading Tubes_IPC<br>■ Disposable Tips & Rack<br>■ Elution Tubes<br>■ Elution Tube Caps<br>■ Waste Tray<br>■ Contamination Shield Filter Paper |

4) Remove the shrink-wrap enclosing both Buffer Cartridges ① and ② within Cleanbench-1.

> ⚠ **Inspect the wells of the Buffer Cartridge and make sure all liquids are at the bottom of the wells.**

5) Take the necessary number of *AccuPower*® Diagnostic Kit tube from the freezer and insert the diagnostic kit tube into the elution tube Rack. Remove the covered foil of diagnostic kit tube. Mark each strip of the diagnostic tubes with the corresponding column number.

> ⚠ **Ensure that the diagnostic tubes are marked so they can be identified during the process.**
>
> ⚠ **At the bottom of the elution tube rack, there is a groove fitted to the *ExiPrep*™ 48 Dx instrument. When viewed from above, place the groove side downwards and insert the premix tubes into two upper rows as shown below figured 61.**

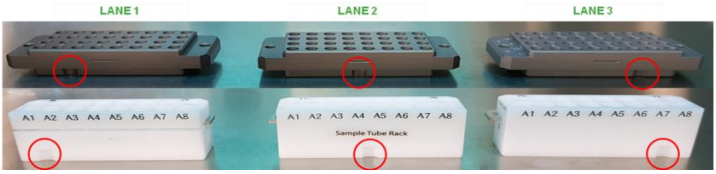



**Fig. 61** Checking the position of Elution Tube Rack and Loading Tube Rack

6) Fasten the protection cover onto the elution tube rack.

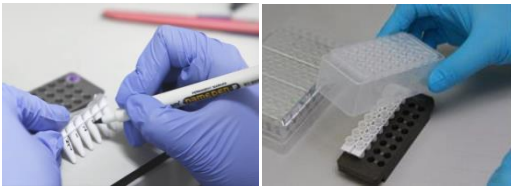
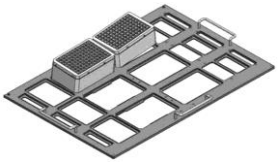
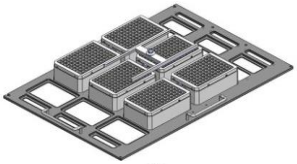


**Fig. 62** Installing protection Cover

7) Open the door of the instrument (*ExiPrep*™ 48 Dx (A-5150)), remove the setup tray installed inside, and place it on a flat experiment bench.

① 

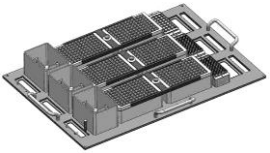
Install buffer cartridge ①, ②
to the sample quantity on the set-up tray.

② 

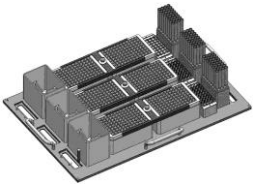
Install clamp on top of the buffer cartridge.
Clamps must be installed per lane and hold the clamp.

③ 

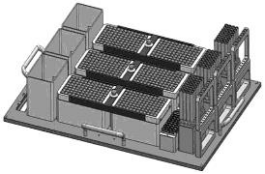
Install the waste tray.

④ 

Install elution tube rack that installed PCR Premix Strip and protection cover to the set-up tray.

⑤ 

Remove the cover of the disposable tip rack and install it on the setup tray.

⑥ 

Install the 8-hole punch.

8) Completed installing components for nucleic acid extraction, prepare control, and samples.

9) Prepare clinical samples in BSC (Class II,III). Before using clean the BSC on which the nucleic acid extraction will be performed. Perform sample within a negative pressure BSC, clean the BSC before using.

> ⚠ **Clean the surface with 0.5% sodium hypochlorite and 70% ethanol or DI water before and after use to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.**
> ⚠ **It must be turn off the UV lamp while using the clean bench.**

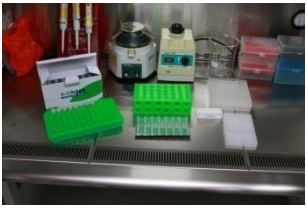


**Fig. 63** Necessary components preparing for sample loading

10) Take the necessary number of Sample Loading Tubes, mark the name on the sample loading tube to prevent confusion. Insert them into the rack.

> ⚠ **Before using a Sample Loading Tube, the bottom of the Sample Loading Tube MUST BE checked for Yellow color (Dried IPC for RNA)**

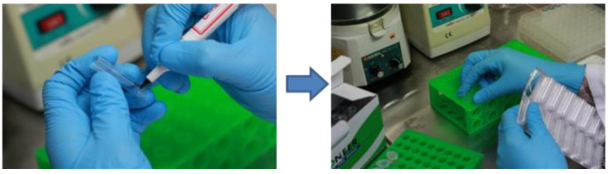


**Fig. 64** Preparing Sample Loading Tube

11) Prepare of container for sample and control (SL buffer, SPC, LPC/HPC), perform to loading into sample loading tube follow next 12) ~ 15) step.

12) Add 400 µL of NTC into a tube that is assigned as NTC. (supplied with *AccuPower*® Diagnostic Kit)

13) Additionally, add 400 µL SPC1~5 into the appropriate SPC wells (supplied with the *AccuPower*® Diagnostic Kit).

> ⚠ **For the pre-date of same lots of Diagnostic kit and Extraction kit, SPC calibration may be skipped. By the Standard, information save automatically, in this case, NTC, LPC, and HPC role as control.**
>
> **When the assay is repeated with the same lot of Diagnostic kit and Extraction kit**
> **NTC**: Load SL buffer **400 µL in NTC tube.**
> **LPC**: Load LPC **400 µL** (**blue cap tube**, the component of *AccuPower*® Diagnostic Kit)
> **HPC**: Load HPC **400 µL** (**red cap tube**, the component of *AccuPower*® Diagnostic Kit)

14) Ready to use the Sample loading tube loaded product's control, install the Sample Tube Rack

> ⚠ **After unlocking the sample tube rack's fixing device, set the tube.**
> ⚠ **When the Sample Tube Rack installs, keep vertical direction during removal and installation of the rack to prevent the pour of loaded solution.**

15) Load 400 µL of clinical sample to Sample Loading Tube. Finish the clinical sample loading, move the Sample Loading Tube to Sample Tube Rack.

> ⚠ **Confirm the exact position of each Sample Loading Tube, and then set up.**
> ⚠ **If a clinical sample contaminates gloves or tips, remove the pollutant immediately, then use a new one.**
> ⚠ **Once the tube has been installed, push the fixing device to lock Sample Loading Tube's position.**

**Fig. 65** The locker to hold the Sample Loading Tube

16) Place the Sample Tube Rack on *ExiPrep*™ 48 Dx setup tray.

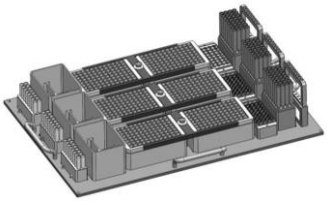


**Fig. 66** Install of Sample Tube Rack

17) Check all components are installed, normally on Setup tray.

18) Install the Setup tray on the *ExiPrep*™ 48 Dx instrument.

> ⚠ **Check each side, left: Sample Tube Rack / Right: 8-hole punch, then push the Setup tray into the instrument, carefully.**

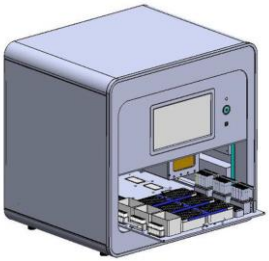


**Fig. 67 Install of Setup tray**

19) Finish all process – setting the program, ready to sample and install the setup tray- Click the "Apply Run" screen located right bottom to start to extract the nucleic acid.

> ⚠ **Running time of extraction takes 60~80 minutes according to sample type.**
> ⚠ **If an error message occurs during the extraction process, please contact the nearest store or the BIONEER International Molecular Diagnosis TS team.**


**Fig. 68 Start extraction of nucleic acid through *ExiPrep*™ 48 software**

## Part 3. Running *ExiPrep*™ 48 Dx and *Exicycler*™ 96 using *ExiPrep*™48 software

*** Please refer to the Equipment User Guide for basic instructions on using *Exicycler*™96 and *ExiPrep* ™ 48 software.**

1) Finish nucleic acid extraction, the pop-up message to notify the end. Press the “Door” button to open the door on the front of the machine, and take out the Setup Tray.

> ⚠ **Finish the extraction of nucleic acid, take out the Setup Tray within 10 minutes. Then separate the PCR Premix Strip from Elution Tube Rack, the process after steps. The long delay can lead to the degradation of nucleic acid, which may affect the result value.**

2) Refer to 8.4 Experimental procedure Ⅰ-Part 3. 4)~7), Ready to PCR process after separate the PCR Premix Strip of Elution Tube Rack.

3) Click the “Assign” icon on the main screen of *ExiPrep*™48 software.
‘Assign icon’ consist of six tabs.

| | |
|---|---|
| **Assign** | Assign the Prep WorkList on 96 well plates, marked the strip number. |
| **Current Step** | It indicates the progress of nucleic acid extraction in *ExiPrep*™48 Dx. Prep: middle of nucleic acid extracting / Prep End: Finish the nucleic acid extraction |
| **Diagnosis Kit** | In Prep WorkList, displayed the diagnostic kit that has been extracted. The selected diagnostic kit can operate PCR with other diagnostic kits at the same time. |
| **Prep Kit** | It indicates the used extraction kit in prep WorkList. |
| **Start Time** | It indicates the start time of nucleic acid extraction. |
| **Finish Time** | It indicates the finish time of nucleic acid extraction. |

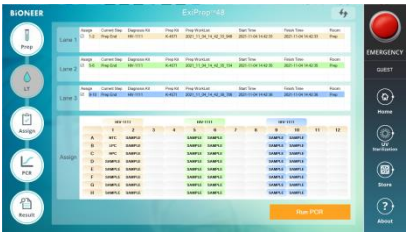



**Fig. 69 PCR Random Access**

4) Click the “Assign” icon. The information list of finished nucleic acid extraction appears on the screen. Select the box for the desired PCR process. Decide the PCR well position according to lane position of *ExiPrep*™ 48 Dx.


**Fig. 70 Select the sample for PCR process**

5) Press the Door button of *Exicycler*™ 96 for 2 seconds, and the 96-well thermal block will get out of the instrument. Set PCR Premix Strip the right position selected by the software.

> ⚠ **PCR Premix Strip position exactly matches with the assigned position in software.**
> ⚠ **When running PCR under 4 strips, put the balance strip in opposite position to balance of *Exicycler*™ 96 thermal block.**

6) After the PCR Premix Strip setting, press the “Run PCR” button located in the lower right. A pop-up window “Data name” appears, then fill in the test name, then press the “OK” button.

> ⚠ **WorkList saves this way ; *ExiPrep*™ 48 software> SET UP > Data > WorkList**

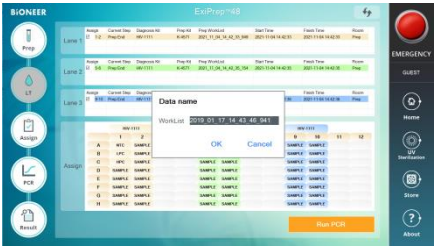



**Fig. 71 Pop-up window of “Data name”**

7) Complete 6) step, *Exicycler*™ 96 runs automatically.

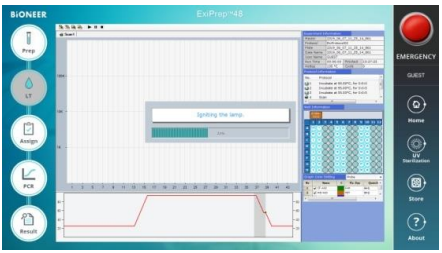



**Fig. 72 PCR Running screen**

8) Complete the PCR, Click the 'Result' icon to confirm the result.

| | |
|---|---|
| ⚠ | **Click "Analysis", an analysis program appears in a pop-up window and can confirm detailed result.** |
| ⚠ | **After clicking the "Print" button (right of Analysis button), select the target analysis result to print can print as a report.** |
| ⚠ | **Analysis result saves automatically on this folder.** |
| ⚠ | ***ExiPrep*™ 48 software> SET UP > Data > WorkList > relevant data.** |


**Fig. 73 Data analysis**

## 8.6 Handling process of experimental waste

### 8.6.1 *ExiPrep*™ 16 Dx

1) Remove all consumables and components, starting with the Buffer Cartridges and various racks from the instrument, and discard all liquids and consumables in their appropriate containers.

> ⚠ **If un-used wells are present in the Buffer Cartridges, take a lint-free cloth or 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the lids on the Buffer Cartridges and keep them in a Cleanbench-1 for later use.**
>
> ⚠ **Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedures.**

2) Press the “Misc Set” button, remove Tip Protector and Contamination Shield, then Cleaning with 70% ethanol and press the ‘Misc Set’ button again

3) Push the Base Plate in, shut the instrument door, and initiate UV sterilization by clicking “UV ON” on the control panel.

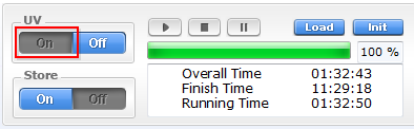



**Fig. 74** ***ExiPrep***™**16 Dx control panel - UV**

### 8.6.2 *ExiPrep*™ 48 Dx

1) Remove all consumables and components, starting with the Buffer Cartridges and various racks from the instrument, and discard all liquids and consumables in their appropriate containers.

> ⚠ **If un-used wells are present in the Buffer Cartridges, remove the used Filter tip from Buffer Cartridge ②. Take a lint-free cloth or 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the lids on the Buffer Cartridges and keep them in a Cleanbench-1 for later use.**
>
> ⚠ **Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedures.**

2) Remove Tip Protector and Contamination Shield, then Cleaning with 70% ethanol and reinstall.

3) Push the Setup tray in, shut the instrument door, and initiate UV sterilization by clicking “UV ON” on the control panel.

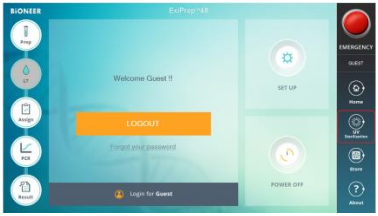



**Fig. 75** ***ExiPrep***™**48Dx control panel - UV**

### 8.6.3 *Exicycler*™**96**

1) After the PCR run is finished, select the ‘Result’ tab to check the results of each sample.

> ⚠ **Do not peel off an optical sealing firm from Diagnostic Kit. discard them according to local safety regulations or internal laboratory procedure**

## 8.7 Data Analysis

**(1) Calibration (HIV-1 SPC (1) – (5))**

For the test with a new Lot of diagnostic kit and extraction kit, calibration must be performed. The test uses 5 wells of SPC (HIV-1 SPC (1) to (5)) to generate a standard curve. Additionally, the user can check for batch validity with *ExiStation*™ manager software either by the monitor or in a printed report.

**(2) Control (HIV-1 LPC and HPC)**

Every test is provided with controls. The test uses 2 wells of PCs (HPC, LPC) to confirm the validity of each test. The user can check the validity of the test with *ExiStation*™ manager software either by the monitor or in a printed report.

**(3) NTC**

Every test uses 1 well of NTC to check any contamination in the process of sample loading, nucleic acid extraction, PCR preparation in order to prevent false-positive error.

The validity of SPC and NTC are determined by the Ct value of the HIV-1 signal. If the assay is valid, HIV-1 Ct will be 'undetermined' in NTC well and the SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User Guide, section 10. Troubleshooting.

**Table 3. Specimen results are interpreted as follows:**

| Titer Result (IU/mL*) | Interpretation |
|---|---|
| Not detected | No Ct value (>45Ct) of HIV-1 was obtained. Results are reported as "Not detected." |
| <5.00E+01IU/mL (2.73E+01cp/mL) | Calculated IU/mL are below the Limit of Quantification of the assay. Report results as "<5.00E+01 IU/mL (2.73E+01cp/mL)". |
| ≥5.00E+01 IU/mL (2.73E+01cp/mL) and ≤1.00E+08 IU/mL (5.46E+07cp/mL) | Calculated results greater than or equal to 5.00E+01 IU/mL(2.78E+01cp/mL) and less than or equal to 1.00E+08 IU/mL(5.46E+07cp/mL) are within the Linear Range of the assay. |
| >1.00E+08 IU/mL (5.46E+07cp/mL) | Calculated IU/㎖ are above the range of the assay. Results are reported as "greater than 1.00E+08 IU/mL (5.46E+07cp/mL) ". If quantitative results are desired, the original specimen should be diluted with HIV-1-negative human EDTA-plasma and the test repeated. Multiply the reported result by the dilution factor. |

**The conversion factor between HIV-1 RNA cp/mL and HIV-1 IU/mL is 0.55 cp/IU(1.83IU/cp).**

## 8.8 Quality Control

**(1) IPC (Internal Positive Control)**

Every test tube contains an IPC to check PCR inhibition by the impurity or the mismanaged thermal cycling to monitor the whole process. IPC is dried within Sample Loading tube (accessory for nucleic acid extraction, not provide).

High concentrations of HIV-1 RNA can lead to a reduced or absent fluorescence signal of the IPC due to PCR competition. The validity of IPC is determined by the Ct value of the IPC signal.

If the Ct value is within a specified range, it is valid. If the Ct value is out of the specified range, it is invalid. The Ct value of the HIV-1 signal determines the validity of SPC and NTC.

If the assay is valid, HIV-1 Ct will be “undetermined” in NTC well, and the SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User Guide section 10. Troubleshooting.

The result of IPC determines the validity of the test and the Ct value of the HIV-1 signal determines the HIV-1 concentration (IU/mL) of the sample. For the high titer specimen above the desired quantitative range, the original specimen should be diluted with the SL buffer provided, and the test must be repeated.

# 9. PERFORMANCE CHARACTERISTIC

## 9.1 Analytical Characteristics

### 9.1.1 Limit of Detection (LoD)

The limit of detection of *AccuPower*® HIV-1 Quantitative RT-PCR Kit was determined by analysis of serial dilutions of the WHO International Standard for HIV-1 RNA for Nucleic Acid Amplification Technology Assays (3rd WHO International Standard), in HIV-negative human EDTA plasma Panels of 7 dilutions levels plus a negative were tested with 3 lots of *AccuPower*® HIV-1 Quantitative RT-PCR Kit.

*AccuPower*® HIV-1 Quantitative RT-PCR Kit detected HIV-1 RNA with a detection rate of 95%, as determined by PROBIT, at a concentration of 33.1 IU/mL(18.09 cp/mL).

**Table 4. Detection rate of *AccuPower*® HIV-1 Quantitative RT-PCR Kit at each concentration**

| Nominal concentration | | | | Number of replicates tested (N) | Number of positive detected (N) | Positive Rate (%) |
|---|---|---|---|---|---|---|
| IU/mL | $Log_{10}$ IU/mL | cp/mL | $Log_{10}$ cp/mL | | | |
| **NTC** | 0.00 | 0.00 | 0.00 | 72 | 0 | 0% |
| **3.125** | 0.49 | 1.71 | 0.24 | 72 | 21 | 29% |
| **6.25** | 0.80 | 3.42 | 0.54 | 72 | 38 | 52% |
| **12.5** | 1.10 | 6.83 | 0.83 | 72 | 58 | 80% |
| **25** | 1.40 | 13.66 | 1.14 | 70 | 65 | 92% |
| **50** | 1.70 | 27.32 | 1.44 | 72 | 70 | 97% |
| **100** | 2.00 | 54.64 | 1.74 | 72 | 72 | 100% |

**Table 5. Limit of Detection Probit Analysis in EDTA plasma**

| Concept | LoD by Probit analysis at 95% detection rate | 95% Confidence interval |
|---|---|---|
| **IU/mL (cp/mL)** | 33.1 (18.09) | 24.5(13.6) – 44.7(24.8) |
| **$Log_{10}$ IU/mL ($Log_{10}$ cp/mL)** | 1.52 (1.26) | 1.39(1.13) – 1.65(1.39) |

### 9.1.2 Traceability

The Traceability study of the *AccuPower*® HIV-1 Quantitative RT-PCR was determined by testing the WHO 3rd HIV-1 International Standard panel (NIBSC code:10/152, UK) containing 5.27 $Log_{10}$ IU/mL of HIV-1, subtype B and HIV-1 Standard Positive Control and virus particle (ATCC85E) three dilutions of international standard panel, 7dilutions of Standard Positive Control and one dilution (8 $log_{10}$ IU/mL) of virus particle was tested.

All material demonstrated co-linear dilution performance across the linear range of *AccuPower*® HIV-1 Quantitative RT-PCR Kit. According to these results, quantification value for HIV-1 international standard positive panel, Standard Positive Control and virus particle was similar to the expected value with the results of Deviation from linearity value within 0.2 $log_{10}$ IU/mL.

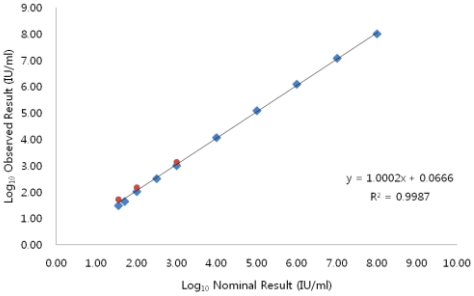



**Fig. 76 Traceability to WHO international standard panel**

### 9.1.3 Verification of limit of detection for group M subtypes, group O and group N

The verification of limit of detection of *AccuPower*® HIV-1 Quantitative RT-PCR Kit detection for group M subtypes, group O and group N was determined by analysis of 3 different dilutions levels of the 2nd WHO International Reference Panel (NIBSC code: 12/224, UK) and 1st WHO International Reference Panel HIV-1 CRF's (NIBSC code: 13/214, UK) and seracare HIV RNA Genotype reference panel (PRD202) in EDTA-plasma (Seracare, Milford, USA).
24-replicate was performed in each dilution and the study results demonstrate that the *AccuPower*® HIV-1 Quantitative RT-PCR Kit was verified to detect HIV-1 RNA in EDTA-plasma at a concentration as low as 1.54 $Log_{10}$IU/mL (1.28 $Log_{10}$cp/mL), with a positivity rate greater than or equal to 95%.

Table 6. Detection of *AccuPower*® HIV-1 Quantitative RT-PCR Kit at each concentration

| Group | Subtype | Concentration | | Number of replicates tested (N) | Number of Positive detected (N) | Positive Rate (%) |
|---|---|---|---|---|---|---|
| | | $Log_{10}$ IU/mL | $Log_{10}$ cp/mL | | | |
| M | A | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 24 | 100 |
| | | 1.24 | 0.98 | 24 | 21 | 88 |
| | C | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 19 | 79 |
| | D | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 16 | 67 |
| | F | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 16 | 67 |
| | G | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 19 | 79 |
| | CRF01 AE | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 12 | 50 |
| | CRF01 AG | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 24 | 100 |
| | | 1.24 | 0.98 | 24 | 20 | 83 |
| | H | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 19 | 79 |
| Group O | | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 20 | 83 |
| Group N | | 1.85 | 1.59 | 24 | 24 | 100 |
| | | 1.54 | 1.28 | 24 | 23 | 96 |
| | | 1.24 | 0.98 | 24 | 21 | 88 |

### 9.1.4 Linear range and Limit of Quantification (LoQ)

Linearity and LoQ of main subtype B were performed with a dilution series of the WHO 3rd HIV-1 International Standard panel (NIBSC code: 10/152, UK) for low titer members and HIV-1 Virus Particle (ATCC 85E) for high tier member, is test with *AccuPower*® HIV-1 Quantitative RT-PCR Kit.

Nine (9) dilutions of each panel from 8 $log_{10}$IU/mL to 1.7 $log_{10}$IU/mL (1.44$Log_{10}$cp/mL) for HIV-1 Group M(subtype B) was prepared and three (3) dilution of each panel from 3.0 $log_{10}$ IU/mL (2.74$Log_{10}$cp/mL) to 1.7 $log_{10}$ IU/mL (1.44$Log_{10}$cp/mL) for another Group M subtype, Group N and O was prepared.

The evaluation of main LoQ and Linearity was performed with three (3) different of *AccuPower*® HIV-1 Quantitative RT-PCR Kit. Test was performed with each concentration two (2) replicates and two (2) runs per day and on four (4) different days, on three (3) different *ExiStation*™ system instruments, resulting in forty-eight (48) overall data points per dilutions.

Linear range claim for *AccuPower*® HIV-1 Quantitative RT-PCR Kit was from 1.70 $Log_{10}$IU/mL (1.44$Log_{10}$cp/mL) to at least 8.00 $Log_{10}$IU/mL (7.74$Log_{10}$cp/mL), with maximum deviation between the observed mean $Log_{10}$ titer and the best fitted $1^{ST}$-order model of less than 0.20 $Log_{10}$ for each concentration level tested in this interval. Therefore, the results of this study support the claimed linear range of 1.70 to 8.00 $Log_{10}$IU/mL(1.44 $Log_{10}$cp/mL to 7.74 Log $_{10}$cp/mL).

At a concentration of 1.70 $Log_{10}$ IU/mL (1.44 $Log_{10}$cp/mL), it was included in the total analytical error (TAE) reference value of 1.00 $Log_{10}$IU/mL (0.74 $Log_{10}$cp/mL). Therefore, the claimed LOQ for the *AccuPower*® HIV-1 Quantitative RT-PCR Kit considering all HIV-1 subtypes is 1.70 $Log_{10}$IU/mL (1.44 $Log_{10}$cp/mL).

**Table 7. Linear equation and range of all HIV-1 subtypes analyzed**

| HIV-1 Subtype | Linear equation in genotype linearity study | Maximum difference between HIV-1 subtype B and corresponding HIV-1 subtype ($Log_{10}$ IU/mL) | Linear range |
|---|---|---|---|
| **A** | y = 1.053x - 0.007 | -0.18 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **B** | y = 1.006x - 0.104 | Not applicable | 1.70 $Log_{10}$IU/mL to 8.00 $Log_{10}$ IU/mL |
| **C** | y = 1.090x - 0.181 | -0.07 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **D** | y = 1.092x - 0.214 | -0.04 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **F** | y = 1.034x - 0.088 | -0.06 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **G** | y = 1.109x - 0.237 | -0.04 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **CRF AE** | y = 1.080x - 0.175 | -0.05 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **CRF AG** | y = 1.087x - 0.133 | -0.11 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |
| **H** | y = 1.101x - 0.272 | 0.01 | 1.70 $Log_{10}$IU/mL to 3.00 $Log_{10}$ IU/mL |

Table 8. LOQ of all HIV-1 subtypes analyzed (IU/mL and cp/mL results)

| HIV-1 Subtype | Nominal concentration ($Log_{10}$ IU/mL) | N | Average Measured Concentration ($Log_{10}$IU/mL) | Bias ($Log_{10}$ IU/mL) | SD ($Log_{10}$ IU/mL) | TAE = \|Bias\| +2 x SD ($Log_{10}$ IU/mL) | SQRT[2] x2x SD ($Log_{10}$IU/mL) |
|---|---|---|---|---|---|---|---|
| A | 1.70 | 28 | 1.74 | 0.04 | 0.32 | 0.69 | 0.92 |
| C | 1.70 | 28 | 1.70 | 0.00 | 0.34 | 0.68 | 0.97 |
| D | 1.70 | 28 | 1.63 | -0.07 | 0.33 | 0.74 | 0.94 |
| F | 1.70 | 28 | 1.63 | -0.07 | 0.28 | 0.63 | 0.8 |
| G | 1.70 | 28 | 1.65 | -0.04 | 0.34 | 0.73 | 0.97 |
| CRF AE | 1.70 | 28 | 1.65 | -0.05 | 0.34 | 0.73 | 0.96 |
| CRF AG | 1.70 | 28 | 1.66 | -0.04 | 0.32 | 0.69 | 0.92 |
| H | 1.70 | 28 | 1.54 | -0.16 | 0.32 | 0.81 | 0.92 |
| N | 1.70 | 24 | 1.72 | 0.02 | 0.25 | 0.51 | 0.69 |
| O | 1.70 | 24 | 1.61 | -0.09 | 0.25 | 0.60 | 0.71 |

| HIV-1 Subtype | Nominal concentration ($Log_{10}$ cp/mL) | N | Average Measured Concentration ($Log_{10}$ cp/mL) | Bias ($Log_{10}$ cp/mL) | SD ($Log_{10}$ cp/mL) | TAE = \|Bias\| +2 x SD ($Log_{10}$ cp/mL) | SQRT[2] x2x SD ($Log_{10}$cp/mL) |
|---|---|---|---|---|---|---|---|
| A | 1.44 | 28 | 1.48 | 0.04 | 0.06 | 0.43 | 0.66 |
| C | 1.44 | 28 | 1.44 | 0.00 | 0.08 | 0.42 | 0.71 |
| D | 1.44 | 28 | 1.37 | -0.07 | 0.07 | 0.48 | 0.68 |
| F | 1.44 | 28 | 1.37 | -0.07 | 0.02 | 0.37 | 0.54 |
| G | 1.44 | 28 | 1.39 | -0.05 | 0.08 | 0.47 | 0.71 |
| CRF AE | 1.44 | 28 | 1.39 | -0.05 | 0.08 | 0.47 | 0.70 |
| CRF AG | 1.44 | 28 | 1.40 | -0.04 | 0.06 | 0.43 | 0.66 |
| H | 1.44 | 28 | 1.28 | -0.16 | 0.06 | 0.55 | 0.66 |
| N | 1.44 | 24 | 1.46 | -0.02 | 0.01 | 0.25 | 0.43 |
| O | 1.44 | 24 | 1.35 | -0.09 | 0.01 | 0.34 | 0.45 |

### 9.1.5 Precision

Precision claim for *AccuPower*® HIV-1 Quantitative RT-PCR Kit was determined by analysis of international standard panel (3rd WHO International Standard) and HIV-1 virus particle (ATCC 85E). 11 dilution levels were tested in eighty (80) replicates for each level across three lots of *AccuPower*® HIV-1 Quantitative RT-PCR Kit using *ExiStation*™ system for twenty (20) days (for repeatability) and three (3) dilution of International standard panel were tested in One hundred forty (140) replicates for each level for five (5) days (for reproducibility).

**Table 9. The Summary Results of Repeatability**

| Nominal concentration ($Log_{10}$ IU/mL) | Assigned concentration ($Log_{10}$ IU/mL) | No. of valid tests | Within-Run ($S_r$) | Between-Run ($S_{rr}$) | Between-Day ($S_{dd}$) | Total Precision ($S_T$) |
|---|---|---|---|---|---|---|
| 8.00 | 8.10 | 80 | 0.06 | 0.02 | 0.04 | 0.07 |
| 7.00 | 7.08 | 80 | 0.06 | 0.02 | 0.05 | 0.08 |
| 6.00 | 6.07 | 80 | 0.04 | 0.03 | 0.03 | 0.06 |
| 5.00 | 5.04 | 80 | 0.06 | 0.01 | 0.05 | 0.08 |
| 4.00 | 4.08 | 80 | 0.08 | 0.01 | 0.05 | 0.09 |
| 3.00 | 3.08 | 80 | 0.09 | 0.05 | 0.07 | 0.13 |
| 2.70 | 2.79 | 80 | 0.15 | 0.03 | 0.07 | 0.17 |
| 2.60 | 2.71 | 80 | 0.16 | 0.06 | 0.08 | 0.19 |
| 2.48 | 2.58 | 80 | 0.16 | 0.09 | 0.07 | 0.19 |
| 2.30 | 2.35 | 80 | 0.20 | 0.10 | 0.06 | 0.23 |
| 2.00 | 2.01 | 80 | 0.29 | 0.14 | 0.11 | 0.34 |

| Nominal concentration ($Log_{10}$ cp/mL) | Assigned concentration ($Log_{10}$ cp/mL) | No. of valid tests | Within-Run ($S_r$) | Between-Run ($S_{rr}$) | Between-Day ($S_{dd}$) | Total Precision ($S_T$) |
|---|---|---|---|---|---|---|
| 7.74 | 7.84 | 80 | 0.09 | 0.03 | 0.03 | 0.09 |
| 6.74 | 6.82 | 80 | 0.06 | 0.03 | 0.02 | 0.07 |
| 5.74 | 5.81 | 80 | 0.07 | 0.05 | 0.01 | 0.09 |
| 4.74 | 4.78 | 80 | 0.1 | 0.05 | 0.02 | 0.11 |
| 3.74 | 3.82 | 80 | 0.06 | 0.03 | 0.02 | 0.07 |
| 2.74 | 2.82 | 80 | 0.14 | 0.09 | 0.02 | 0.17 |
| 2.44 | 2.53 | 80 | 0.23 | 0.16 | 0.08 | 0.29 |
| 2.34 | 2.45 | 80 | 0.18 | 0.11 | 0.04 | 0.22 |
| 2.22 | 2.32 | 80 | 0.13 | 0.05 | 0.05 | 0.15 |
| 2.04 | 2.09 | 80 | 0.29 | 0.16 | 0.03 | 0.34 |
| 1.74 | 1.75 | 80 | 0.42 | 0.25 | 0.05 | 0.49 |

**Table 10. The Summary Results of Reproducibility**

| Nominal concentration ($Log_{10}$ IU/mL) | Assigned concentration ($Log_{10}$ IU/mL) | No. of valid tests | Standard Deviation (SD) | | | |
|---|---|---|---|---|---|---|
| | | | Between-Lot | Between-Site | Between-Operator | Between-Instrument |
| 3.00 | 2.98 | 140 | 0.15 | 0.11 | 0.14 | 0.09 |
| 2.00 | 1.90 | 140 | 0.32 | 0.30 | 0.31 | 0.30 |
| 1.70 | 1.77 | 140 | 0.24 | 0.19 | 0.25 | 0.20 |

| Nominal concentration ($Log_{10}$ cp/mL) | Assigned concentration ($Log_{10}$ cp/mL) | No. of valid tests | Standard Deviation (SD) | | | |
|---|---|---|---|---|---|---|
| | | | Between-Lot | Between-Site | Between-Operator | Between-Instrument |
| 2.74 | 2.72 | 140 | 0.15 | 0.13 | 0.15 | 0.13 |
| 1.74 | 1.64 | 140 | 0.34 | 0.35 | 0.35 | 0.36 |
| 1.44 | 1.50 | 140 | 0.26 | 0.23 | 0.26 | 0.25 |

### 9.1.6 Interfering substances

Interfering effects by seventeen exogenous substances (included anti-viral substance) and by seven endogenous substances was tested for interfering of *AccuPower*® HIV-1 Quantitative RT-PCR kit. Potentially interfering endogenous and exogenous substances were spiked into EDTA-plasma in the absence or presence of three times the LoD (2.00 $Log_{10}$IU/mL/1.74 $Log_{10}$cp/mL) concentration of HIV-1 and were compared to control EDTA-plasma samples containing no spiked interfering substance. Each concentration level for each interfering substance was tested in twelve replicates.

**Table 11. Interference-Exogenous Interfering Substances**

| No. | Potential interfering substances | Concentration (ug/mL) | No. | Potential interfering substances | Concentration (ug/mL) |
|---|---|---|---|---|---|
| **1** | Acyclovir | 9.80 | **10** | Abacavir | 3.89 |
| **2** | Stavudine | 0.68 | **11** | Ribavirin | 3.57 |
| **3** | Enfuvirtide | 8.60 | **12** | Lamivudinie | 1.20 |
| **4** | Tenofovir | 0.33 | **13** | Indinavir | 11.84 |
| **5** | Ciprofloxacin | 5.40 | **14** | Vanganciclovir | 7.13 |
| **6** | Nevirapine | 2.40 | **15** | Efavirenz | 4.07 |
| **7** | Nelfinavir | 4.80 | **16** | Zidovudine | 2.29 |
| **8** | Saquinavir | 5.21 | **17** | Amprenavir | 7.66 |
| **9** | Ritonavir | 14.80 | | - | |

**Table 12. Interference-Endogenous Interfering Substances**

| No. | Potential interfering substances | Concentration | No. | Potential interfering substances | Concentration |
|---|---|---|---|---|---|
| **1** | EDTA | 540mg/dL | **5** | Cholesterol | 500mg/dL |
| **2** | Citrate | 0.327M | **6** | Albumin | 5g/dL |
| **3** | Heparin | 3KU/dL | **7** | Bilirubin | 25mg/dL |
| **4** | Hemoglobin | 200mg/dL | | - | |

### 9.1.7 Cross reactivity

The following viruses and Bacteria were tested for cross-reactivity of *AccuPower*® HIV-1 Quantitative RT-PCR Kit. Samples were prepared by diluting organisms or DNA/RNA either in HIV-1 negative EDTA-plasma or in HIV-1 spiked EDTA-plasma at concentration (LODx3) and was tested in three replicates.

Negative HIV-1 EDTA-plasma samples for negative were shown negative and HIV-1 positive specimens spiked in cross-reactivity organisms were shown to detect within ±0.43 $Log_{10}$ IU/mL (0.17 $Log_{10}$ cp/mL).

**Table 13. List of potential cross reactivity organism**

| Viruses | | Bacteria |
|---|---|---|
| Hepatitis A virus | Zika Virus | *Mycobacterium gordonae* |
| Hepatitis B virus | Human herpesvirus 6B | *Staphylococcus aureus* |
| Hepatitis C virus | Human herpesvirus 8 | |
| Epstein-Barr Virus | HIV-2 | |
| Cytomegalovirus | Adenovirus type 5 | |
| Human papilloma virus 16 | Dengue virus types 1 | |
| Human papilloma virus 18 | Dengue virus types 2 | |
| BK human polyomavirus | Dengue virus types 3 | |
| Herpes simplex virus 1 | Dengue virus types 4 | |
| Herpes simplex virus 2 | Influenza Virus A(H1N1) | |
| Varicella-Zoster Virus | Influenza Virus A(H3N2) | |
| West Nile Virus | HTLV | |

### 9.1.8 Whole system failure

The Whole System Failure rate was tested with one-hundred two (102) replicates using the *AccuPower*® HIV-1 Quantitative RT-PCR Kit. Positive results were obtained 100% detection of the one-hundred two (102) replicates overall, a system success rate was shown 100% in the *AccuPower*® HIV-1 Quantitative RT-PCR Kit.

**Table 14. Whole System Failure**

| Concentration ($Log_{10}$ IU/mL) | Concentration ($Log_{10}$ cp/mL) | Test number | Detection rate (%) |
|---|---|---|---|
| 2.00 | 1.74 | 102 | 100% |

### 9.1.9 Cross contamination

This evaluation consists of eight (8) samples each of High positive and Negative, and five(5) runs on the same instrument for 5days. All negative samples should not be detecting a HIV-1 signal. The cross-contamination test was performed by using the HIV-1 diagnostic kit according to the CTS guideline. High positive and negative were tested at HLoQ concentration (8.00 $log_{10}$ IU/mL/ 7.74$Log_{10}$cp/mL) and Negative HIV-1 free matrix, respectively.

**Table 15. Cross contamination results**

| Run | Number of samples (Detected / Tested) | | Sample information ($Log_{10}$ IU/mL) | | Sample information ($Log_{10}$ cp/mL) | |
|---|---|---|---|---|---|---|
| | Positive | Negative | 8.00 | Negative | 7.74 | Negative |
| **Run1** | 8/8 | 0/8 | 8.29 | Not detected | 8.03 | Not detected |
| **Run2** | 8/8 | 0/8 | 8.29 | Not detected | 8.03 | Not detected |
| **Run3** | 8/8 | 0/8 | 8.27 | Not detected | 8.01 | Not detected |
| **Run4** | 8/8 | 0/8 | 8.25 | Not detected | 7.99 | Not detected |
| **Run5** | 8/8 | 0/8 | 8.27 | Not detected | 8.01 | Not detected |
| **Average** | | | **8.28** | - | **8.01** | |
| **SD** | | | **0.035** | - | **0.017** | |

**Table 16. Summary of Cross contamination results (Between equipment)**

| Equipment | Number of samples (Detected / Tested) | | Sample information ($Log_{10}$ IU/mL) | | Sample information ($Log_{10}$ cp/mL) | |
|---|---|---|---|---|---|---|
| | Positive | Negative | 8.00 | Negative | 7.74 | Negative |
| **Equipment1** | 8/8 | 0/8 | 8.22 | Not detected | 7.96 | Not detected |
| **Equipment2** | 8/8 | 0/8 | 8.26 | Not detected | 8.00 | Not detected |
| **Equipment3** | 8/8 | 0/8 | 8.26 | Not detected | 8.00 | Not detected |
| **Equipment4** | 8/8 | 0/8 | 8.23 | Not detected | 7.97 | Not detected |
| **Equipment5** | 8/8 | 0/8 | 8.33 | Not detected | 8.07 | Not detected |
| **Average** | | | **8.26** | - | **8.00** | |
| **SD** | | | **0.060** | - | **0.043** | |

## 9.2 Diagnostic Performance Characteristics

### 9.2.1 Sensitivity and Specificity

Total of two-hundred fifty-four (254) HIV-1 positive and Negative EDTA-plasma clinical sample were compared with CE-IVD approved HIV-1 NAT assay.
Diagnostic sensitivity was 96.99% (95% CI 92.52 - 98.82) and the specificity was 100 % (95% CI 96.92 - 100). This satisfies the proposed acceptance criteria of 95% or more.

**Table 17. HIV-1 Clinical evaluation results summary of *AccuPower*® HIV-1 Quantitative RT-PCR Kit**

| | | CE-IVD approved HIV-1 NAT assay | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| ***ExiStation*™ System** | Positive | **129** | **0** | **129** |
| | Negative | **4** | **121** | **125** |
| | Total | **133** | **121** | **254** |

Diagnostic Sensitivity (Percent positive agreement) = 96.99 % (95% C.I 92.52 - 98.82)
Diagnostic Specificity (Percent negative agreement) = 100 % (95% C.I 96.92 – 100)

### 9.2.2 Correlation

*AccuPower*® HIV-1 Quantitative RT-PCR Kit was compared with CE-IVD approved HIV-1 assay. A total one-hundred thirty-three (133) specimens collected from HIV-1 infected patients were tested at an external site ant the results from total one-hundred twenty-nine (129) specimens was analyzed with linear regression method.
The r-squared value was 0.9595, the slope was 0.659 and the intercept was 0.9286 $\log_{10}$ IU/mL.

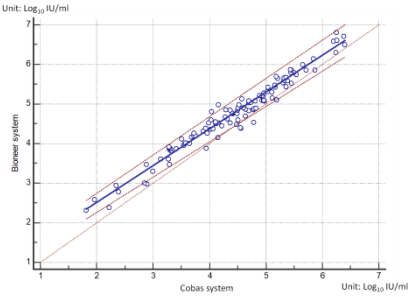



**Fig. 77 Correlation with CE-IVD approved assay.**

### 9.2.3 Verification of precision

Precision was validated by manufacturer. the results of manufacturer`s precision claim was verified in clinical site. This study was analyzed two dilution of HIV-1 international standard panel that was tested with one lot of *AccuPower*® HIV-1 Quantitative RT-PCR Kit according to CLSI EP15-A.2 replicates of each dilution per day was tested at each dilution for 3 days.
The user`s verification results of precision was shown that user`s verification results was lower than manufacturer`s precision claim.
The $S_{within}$ or $S_{Total}$ precision of the *AccuPower*® HIV-1 Quantitative RT-PCR Kit assay was verified to be consistent with the manufacturer's claim.

**Table 18. Summary results of User's Precision Verification**

| | Analytical Performance Precision value | | Verification Performance Precision value | | Verification Performance verification value | |
|---|---|---|---|---|---|---|
| | $\sigma_{within}$ | $\sigma_{total}$ | $S_{within}$ | $S_{total}$ | $S_{within}$ | $S_{total}$ |
| **2.0$Log_{10}$ IU/mL (1.74$Log_{10}$ cp/mL)** | 0.29 (0.21) | 0.34 (0.31) | 0.19 (0.56) | 0.18 (0.82) | 0.45 (0.46) | 0.48 (0.56) |
| **3.0 $Log_{10}$ IU/mL (2.74$Log_{10}$ cp/mL)** | 0.09 (0.25) | 0.13 (0.33) | 0.06 (0.22) | 0.07 (0.33) | 0.14 (0.57) | 0.20 (0.49) |

## 10. TROUBLESHOOTING

| | Comments and suggestions |
|---|---|
| **Internal Positive Control (IPC) invalid results** | |
| If the TAMRA (IPC) Fluorescence signal was not detected in all wells (including controls) | • Extraction and/or PCR configuration error<br>☞ Make sure that the correct extraction/PCR protocol was programmed and performed in accordance with the Kits. Repeat the assay, if necessary. See ***User's Guide* 8. PROTOCOL**<br>• Incorrect extraction or PCR kit use<br>☞ Make sure that you use proper kits for the intended tests.<br>• The kit may have spoiled, due to bad storage or expiration.<br>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See ***User's Guide* 5. STORAGE CONDITION AND SHELF LIFE**<br>• Invalid results.<br>☞ It must be tested with the new reagent |
| If the TAMRA (IPC) Fluorescence signal was not detected in particular wells. | • Inhibition of PCR<br>☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay from the sample pretreatment process which can reduce PCR inhibition.<br>☞ Make sure that you use the validated sample pretreatment method in accordance with the sample type.<br>• Low elution volume due to insoluble material of samples<br>☞ Yield of nucleic acid can be affected by sample conditions (viscosity etc.). Repeat the assay from the sample pretreatment process which can make the sample more soluble. |

| SPC/PC invalid results | |
|---|---|
| If the FAM (SPC) Fluorescence signal was undetermined. | • The kit may have spoiled, due to bad storage or expiration.<br>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.<br>See ***User's Guide*** **5. STORAGE CONDITION AND SHELF LIFE**<br>• Re-use of reagents<br>☞ Make sure not to re-use reagents. Re-use or repeated freeze/thaw cycles of reagents may affect the kit quality and the results of assay conclusively. Repeat the assay with new reagents, if necessary.<br>See ***User's Guide*** **5. STORAGE CONDITION AND SHELF LIFE,**<br>**7. General Precautions**<br>• PCR Protocol error<br>☞ Review your reaction preparation procedure. Confirm the amount of SPC used in a single well.<br>See ***User's Guide*** **8. PROTOCOL**<br>• There may have been a pipetting error.<br>☞ Review the pipetting technique and calibration.<br>• Invalid results.<br>☞ It must be tested with the new reagent |
| **No template Control (NTC) invalid results** | |
| If the FAM fluorescence signal was detected in NTC well. | • Contamination may have occurred.<br>☞ Make sure that work space and instruments are decontaminated and repeat the assay.<br>• The kit may have spoiled, due to bad storage or expiration.<br>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.<br>See ***User's Guide*** **5. STORAGE CONDITION AND SHELF LIFE**<br>• PCR Protocol error<br>☞ Review your reaction preparation procedure. Confirm whether controls and samples are loaded in proper wells which are assigned through S/W protocol (especially NTC well(s)).<br>See ***User's Guide*** **8. PROTOCOL**<br>• There may have been a pipetting error.<br>☞ Review the pipetting technique and calibration. |

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## 12. SYMBOLS

| | | | |
|---|---|---|---|
| REF | Catalog number |  | Temperature limitation |
| IVD | In vitro diagnostic medical device |  | Contains sufficient for test |
|  | Manufacturer |  | Caution, consult accompanying documents |
| LOT | Batch code |  | Expiration date |
|  | Do not reuse |  | Consult instructions for use |
|  | Warning for hazardous and irritation |  | Keep away from sunlight |

## 13. REVISION SUMMARY

| Version | Date | Summary |
|---|---|---|
| 3.9 | 2024-06-13 | • Updated 1. INTENDED USE<br>• Updated 4.1 Contents of the Kit (Added information to identify hazardous substances)<br>• Updated 7. GENERAL PRECAUTIONS<br>• Added 7.1 Product's Limitation<br>• Updated 8.2 Specimen<br>• Updated 8.7 Data analysis |

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