USER'S GUIDE

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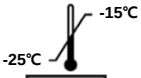
AccuPower®
HIV-1 Quantitative RT-PCR Kit



REF HIV-1211

IVD Quantitative test kit
Human immunodeficiency virus-1 RNA

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BIONEER
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AccuPower® HIV-1 Quantitative RT-PCR Kit

User's Guide

Version No.: 3.7 (2021-09-09)

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 (order@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.

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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. *AccuPower*® HIV-1 Quantitative RT-PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers for monitoring of patient`s prognosis or antiretroviral therapy by measuring HIV-1 viral load. HIV-1 Genotype to group M, N, O within range of 8.0 Log_{10} IU/ml to 1.70 Log_{10} IU/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.

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) 2)]

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)]

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[4) 5)] 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.

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 copies/㎖)	**1300 ㎕ / tube (Green 2㎖ 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 copies/㎖)				1 tube	
	HIV-1 SPC (S3) (400,000 copies/㎖)				1 tube	
	HIV-1 SPC (S4) (4,000,000 copies/㎖)				1 tube	
	HIV-1 SPC (S5) (40,000,000 copies/㎖)				1 tube	
③	**HIV-1 LPC[b]** (4,000 copies/㎖)	**1300 ㎕ / tube (Blue 2㎖ 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 copies/㎖)	**1300 ㎕ / tube (Red 2 ㎖ 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 ㎕ / tube (Clear 2 ㎖ screw tube)**	DEPC-DW, 0.05% Acetylated Bovine serum albumin		3 tubes	Non Template Control
⑤	**SL buffer**	**1300 ㎕ / tube (Clear 2 ㎖ 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	Provide by e-mail or directly

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

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

System	Instrument	Reagent (Extraction)
***ExiStation*™ (A-2200)**	-*ExiPrep*™16 Dx (Cat. No. A-5050) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060)	- *ExiPrep*™ Dx Viral RNA Kit (K-4473) -*ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471) -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) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1)	- *ExiPrep*™ Dx Viral RNA Kit (K-4473) -*ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471) -Sample Loading Tube_RNA IPC (Cat. No. KA-3011)
	Existation™ manager software (Version 4.02.XX)	
***ExiStation*™ 48 (A-2400)** ***ExiStation*™ 48A (A-2410)**	- *ExiPrep*™48 Dx (Cat. No. A-5150) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060-1) -*ExiLT* (Cat.No. A-7100)	- *ExiPrep*™48 Viral DNA/RNA Kit (K-4571) - *ExiPrep*™48 Viral RNA Kit (K-4573) -*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 • Pipette set appropriate volume (1,000 ㎕, 200 ㎕, 20 ㎕ pipette) • Sterilized pipette tips with filters(1,000 ㎕, 200 ㎕, 20 ㎕ tips with filters) • 1.5 ㎖ or 15 ㎖ 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.
- All kit components should be allowed to slowly thaw for at least 10 minutes before initiating an experiment.
- 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.

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 Cleanteches a workspace where filtered air flows outwards, thus keeping a clean environment within the workspace.

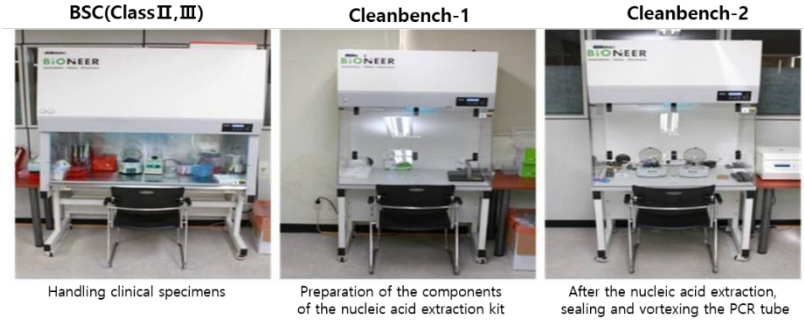


Fig. 1 Biosafety Cabinet (BSC) & Cleanbench

8.2 Specimen

All sample should be treated as potential biohazards. For the best results, RNA extracted from human EDTA-plasma samples is recommended.

8.2.1 Specimen Collection

The *AccuPower*® HIV-1 Quantitative RT-PCR Kit is optimized for RNA extracted from human EDTA-plasma samples. For EDTA-plasma collection, standard specimen collection tubes such as disposable tubes containing EDTA as anticoagulant can be used. All samples should be kept in preservative-free containers.

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.

8.3 Work Flow

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

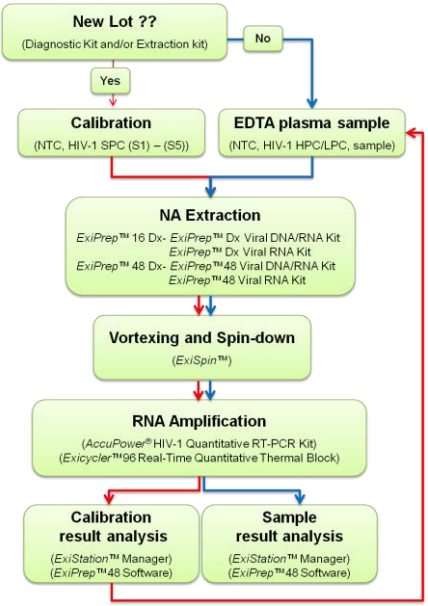


Fig. 2 Work flow

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, refer to the User's Guide.**

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.

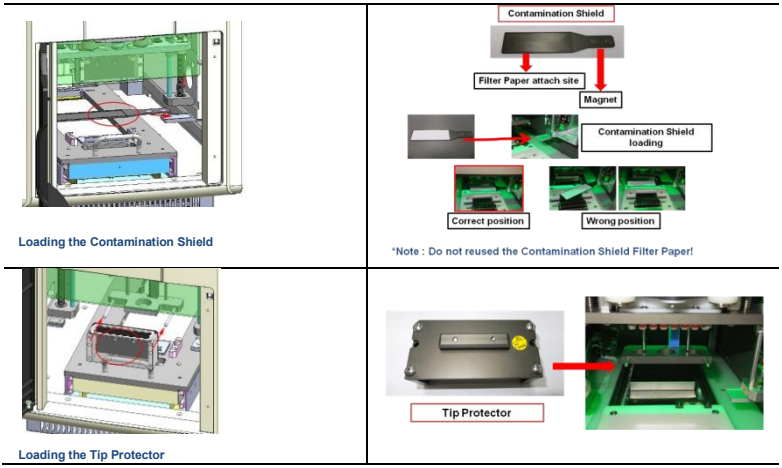


Fig. 6 Location and mounting method of the contamination shield and tip protector

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

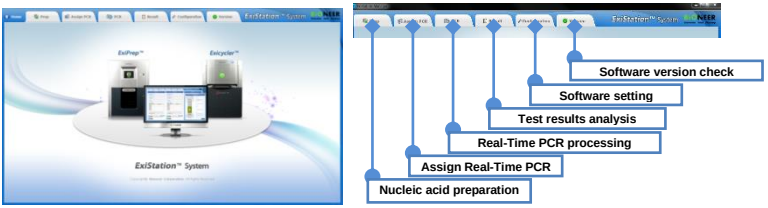


Fig. 8 Tab function of *ExiStation*™ Manager software

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

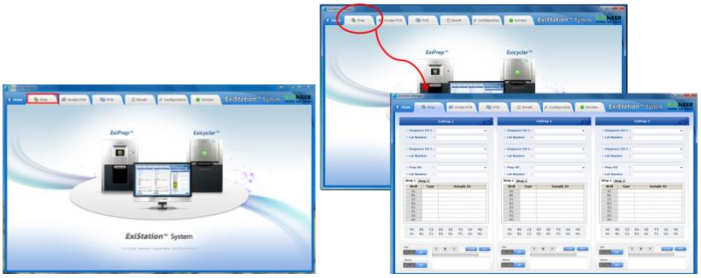


Fig. 9 Prep control panel consist of 5 panels

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

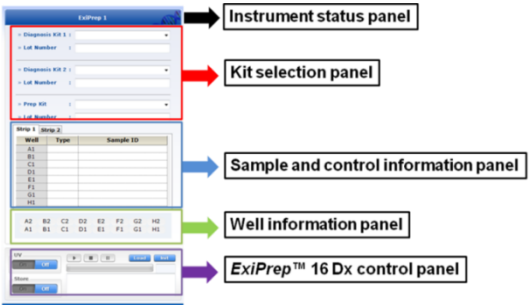


Fig. 10 Prep control panel of *ExiStation*™ Manager software

9) Click the pull-down arrow for “Diagnosis Kit 1”. A popup will appear and select “HIV-1211” from the pull-down menus.

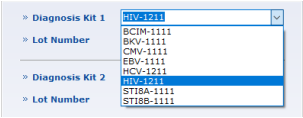


Fig. 11 Selection of Diagnostic kit

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.

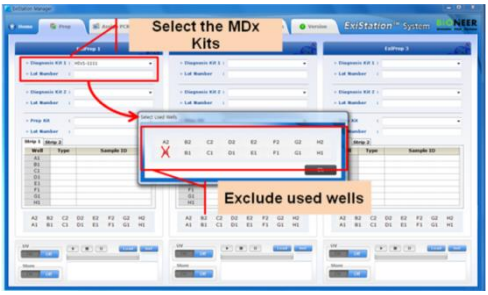


Fig. 12 ‘Prep’ Pop-up window of *ExiStation*™ Manager software

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.

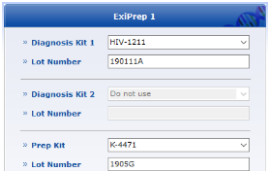


Fig. 13 Entering lot number

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

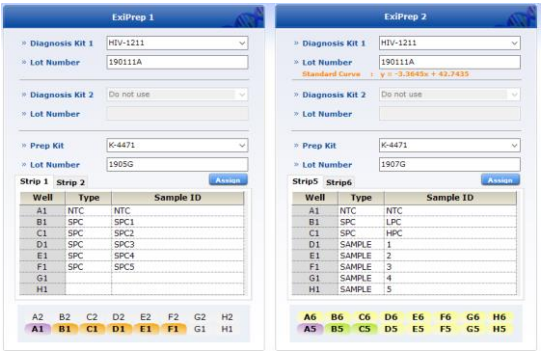


Fig. 14 Enter Sample ID (First assay/Repeated assay)

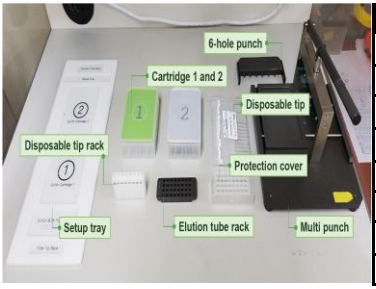
Part 2. Nucleic acid extraction by *ExiPrep*™ 16 Dx

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.**

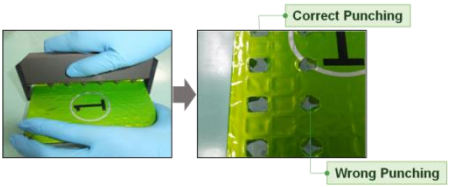


Fig. 17 Punch the film with the Hole Puncher

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

7) Place Buffer Cartridges on the setup tray.

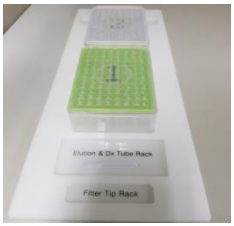


Fig. 18 Install buffer cartridge on the set-up tray

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.**

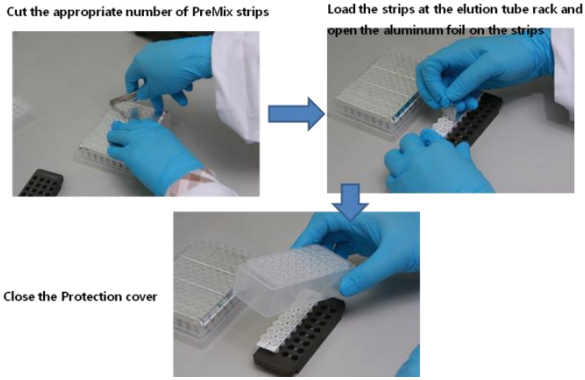


Fig. 19 Inserting the *AccuPower*® Diagnostic Kit tubes into Elution Tube Rack

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.

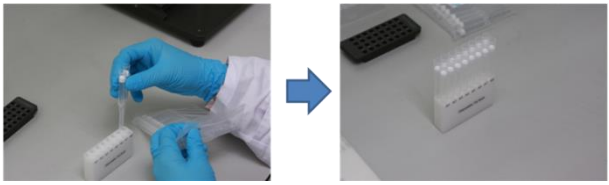


Fig. 20 Load the disposable filter tips at the disposable tip rack

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.**

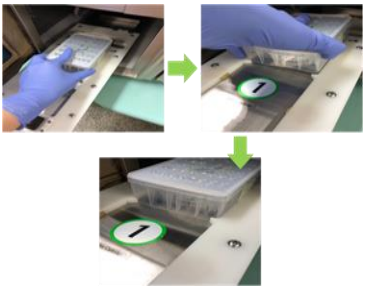


Fig. 22 Place the Buffer Cartridge ②

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.**

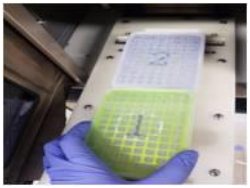


Fig. 23 Place the Buffer Cartridge ①

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.

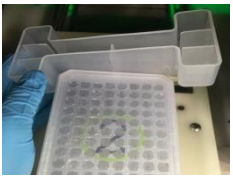


Fig. 25 Loading the Waste tray

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.**

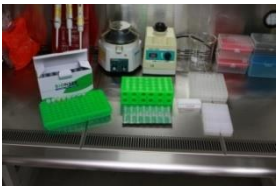


Fig. 26 Necessary components preparing for sample loading

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)**

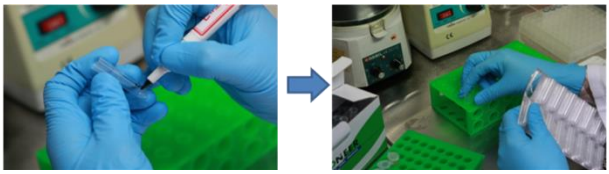


Fig. 27 Preparing Sample Loading Tube

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㎕ of NTC into a tube assigned as NTC (supplied with the *AccuPower*® Diagnostic Kit).

23) Additionally, add 400㎕ 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 save 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㎕ in NTC tube.**
> **LPC**: Load LPC **400㎕** (**blue cap tube**, the component of *AccuPower*® Diagnostic Kit)
> **HPC**: Load HPC **400 ㎕** (**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.**

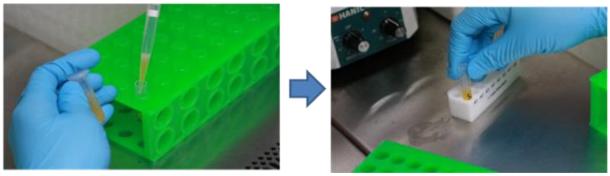


Fig. 28 Load clinical sample to Sample Loading Tube

25) Uncap clinical sample container and pipette 400 ㎕ 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.

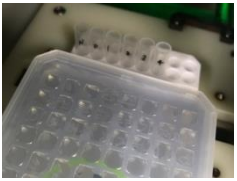


Fig. 30 Install of Sample Tube Rack

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.**

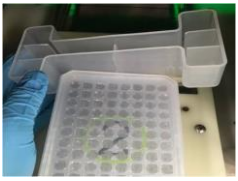


Fig. 31 Re-load Waste Tray

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.**

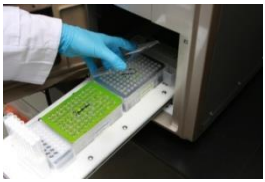


Fig. 32 Remove the lids

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 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

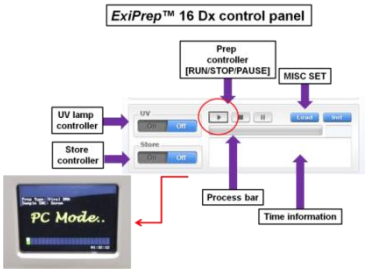


Fig. 34 *Exiprep*™16 Dx control panel

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.**

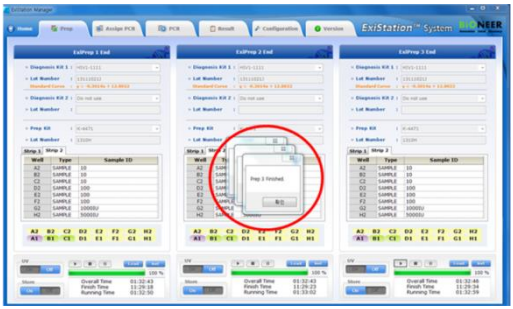


Fig. 35 Pop-up message for extraction finished

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.

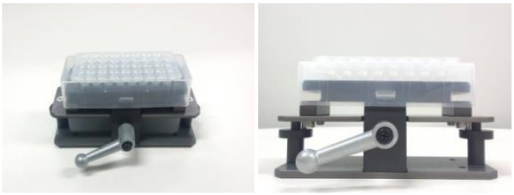


Fig. 37 Picture of Elution Tube Rack on top of Protection Cover Separation Tool

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.

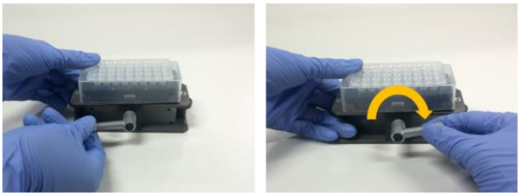


Fig. 38 Picture of lever rotation for fixing Elution Tube Rack to Protection Cover Separation Tool

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.

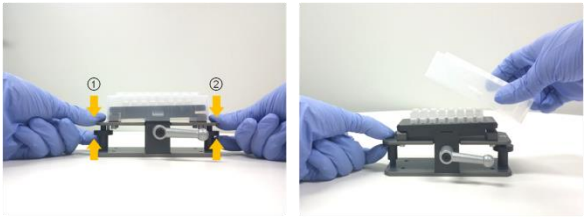


Fig. 39 Picture of pressing down each side of Separation Tool and removing Protection Cover from Separation Tool

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).**

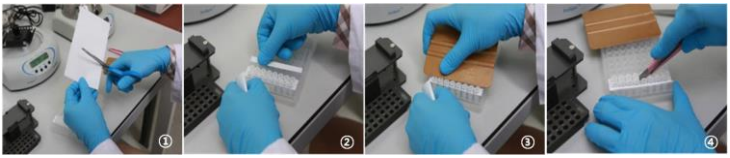


Fig. 40 Seal PCR premix strip

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.**

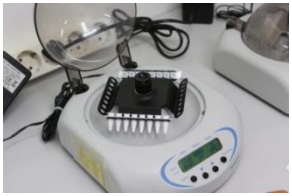


Fig. 41 Mix the PCR Premix Strip using *ExiSpin*™

> ⚠ **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. 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.

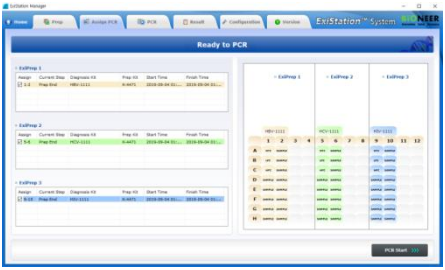


Fig. 43 PCR Random Access

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.

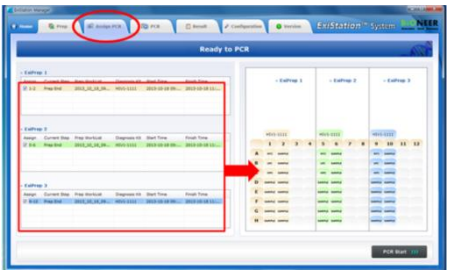


Fig. 44 'Assign PCR' tab - PCR Start

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 in 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.***

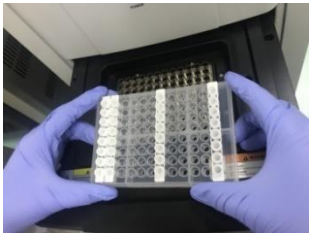


Fig. 45 Way to PCR Premix Strip setup of *Exicycler*™ 96

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'.**

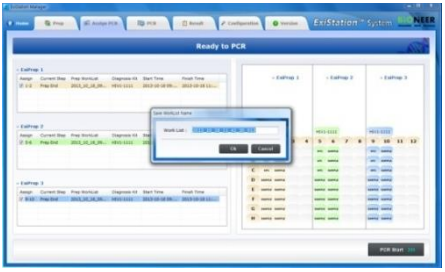


Fig. 46 Pop-up window of "Data name"

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

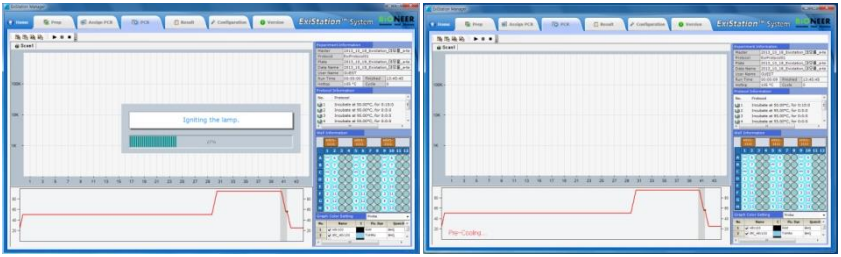


Fig. 47 PCR Running screen

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.

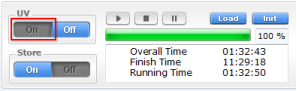


Fig. 48 ExiPrep™16 Dx control panel – UV

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.

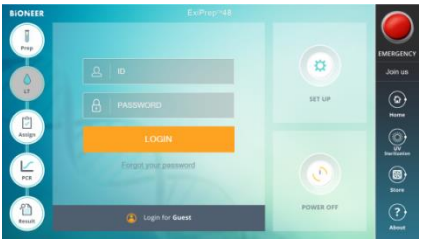


Fig. 50 Main screen of *ExiPrep*™ 48 Dx

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

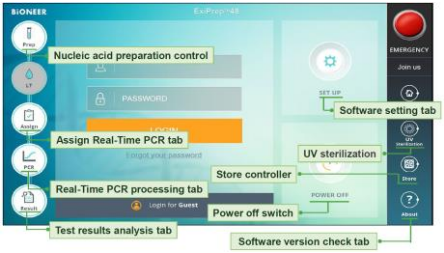


Fig. 51 Icons function of *ExiPrep*™48 software

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.

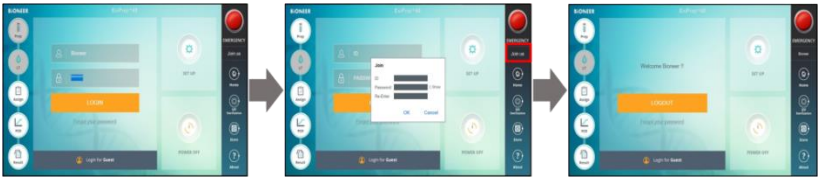


Fig. 53 Decontamination step-2 ; UV sterilization

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-1211.

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.

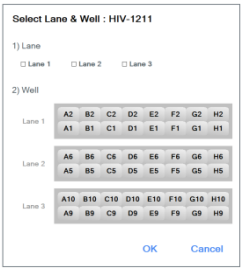


Fig. 56 Screen of 'Select Lane & Well'

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.

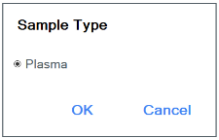


Fig. 58 Screen of ‘Sample Type’

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

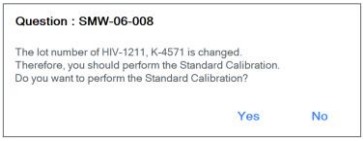


Fig. 59 Pop-up message for Standard Calibration process

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 ■ Hole Punch ■ Sample Tube Rack ■ Elution Tube Rack ■ Clamp	■ Buffer Cartridges ① and ② ■ Sample Loading Tubes_IPC ■ Disposable Tips & Rack ■ Elution Tubes ■ Elution Tube Caps ■ Waste Tray ■ 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.**

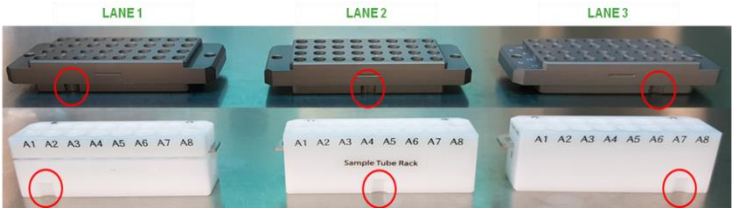


Fig. 61 Checking the position of Elution Tube Rack and Loading Tube Rack

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

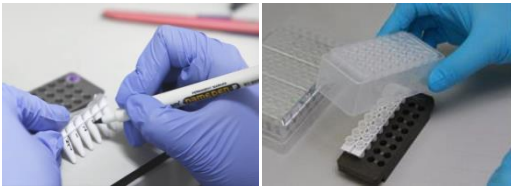
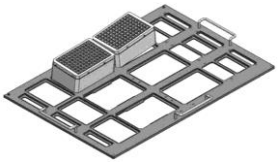
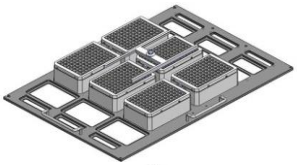


Fig. 62 Installing protection Cover

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 

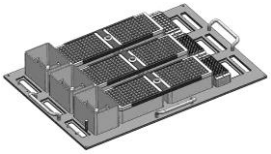
Install buffer cartridge ①, ②
to the sample quantity on the set-up tray.

② 

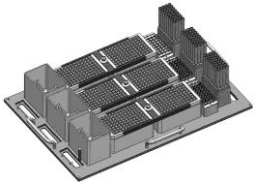
Install clamp on top of the buffer cartridge.
Clamps must be installed per lane and hold the clamp.

③ 

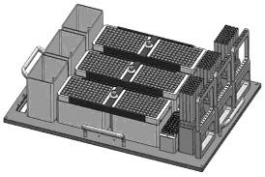
Install the waste tray.

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.**

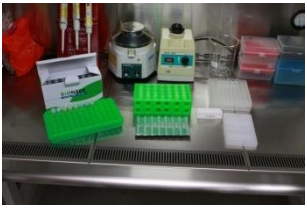


Fig. 63 Necessary components preparing for sample loading

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)**

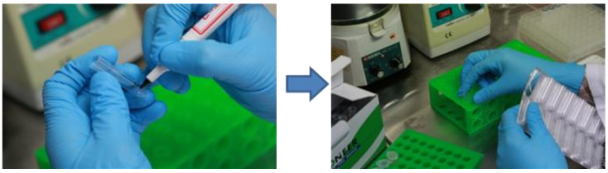


Fig. 64 Preparing Sample Loading Tube

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 $\mu\ell$ of NTC into a tube that is assigned as NTC. (supplied with *AccuPower*® Diagnostic Kit)

13) Additionally, add 400 ㎕ 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㎕ in NTC tube.**
>
> **LPC**: Load LPC **400㎕** (**blue cap tube**, the component of *AccuPower*® Diagnostic Kit)
>
> **HPC**: Load HPC **400 ㎕** (**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 ㎕ 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 hole the Sample Loading Tube

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

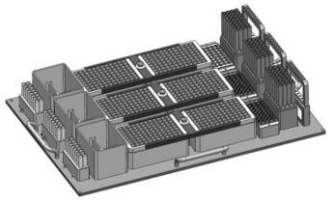


Fig. 66 Install of Sample Tube Rack

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.**

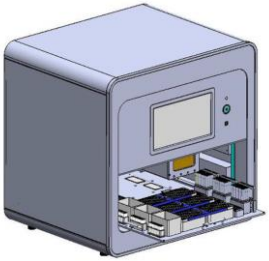


Fig. 67 Install of Setup tray

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 though *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.

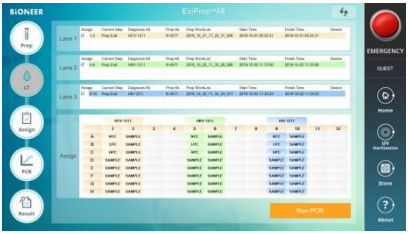


Fig. 69 PCR Random Access

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**

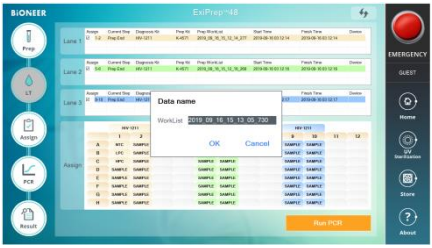


Fig. 71 Pop-up window of “Data name”

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

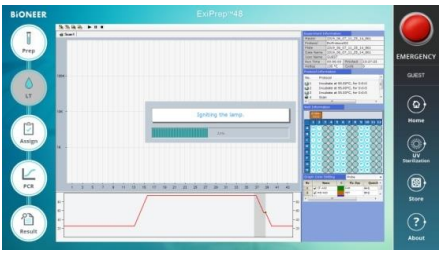


Fig. 72 PCR Running screen

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.**

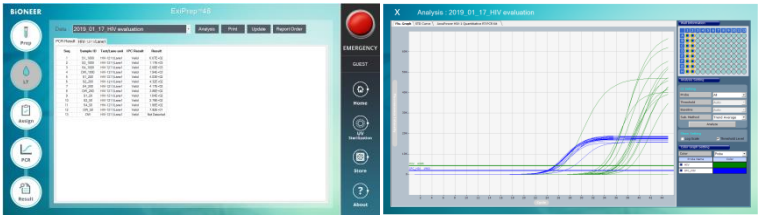


Fig. 73 Data analysis

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.

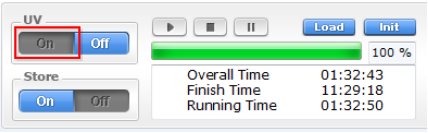


Fig. 74 *ExiPrep*™16 Dx control panel - UV

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. The batch is valid if at least 3 SPCs are valid.

(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/㎖*)	Interpretation
Not detected	No Ct value (>45Ct) of HIV-1 was obtained. Results are reported as "Not detected."
<5.00E+01 IU/㎖	Calculated IU/㎖are below the Limit of Quantification of the assay. Report results as "<5.00E+01".
≥5.00E+01 IU/㎖ and ≤1.00E+08 IU/㎖	Calculated results greater than or equal to 5.00E+01 IU/㎖ and less than or equal to 1.00E+08 IU/㎖ are within the Linear Range of the assay.
>1.00E+08 IU/㎖	Calculated IU/㎖ are above the range of the assay. Results are reported as "greater than 1.00E+08 IU/㎖". 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.

* IU/㎖; HIV-1 RNA concentration in copy/㎖ X 0.72 IU/copy = HIV-1 RNA in IU/㎖

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/㎖) 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/㎖.

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

Nominal concentration		Number of replicates tested (N)	Number of positives detected (N)	Positive rate (%)
IU/ml	Log_{10}IU/㎖			
NTC	0.00	72	0	0%
3.125	0.49	72	21	29%
6.25	0.80	72	38	52%
12.5	1.10	72	58	80%
25	1.40	70	65	92%
50	1.70	72	70	97%
100	2.00	72	72	100%

Table 5. Limit of Detection probit analysis in EDTA plasma

Concept	LoD by PROBIT at 95% detection rate	95% Confidence interval	
IU/㎖	33.1	24.5	44.7
Log_{10} IU/㎖	1.52	1.39	1.65

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.

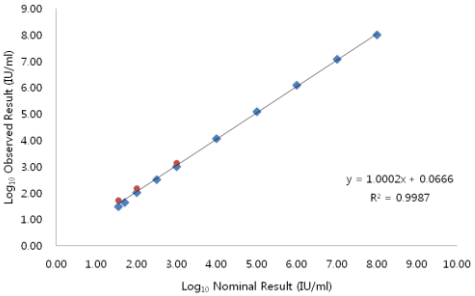


Fig. 76 Traceability to WHO international standard panel

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 dilutions 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, with a positivity rate greater than or equal to 95%.

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

Group	Subtype	Concentration (Log_{10} IU/mℓ)	Number of replicates tested (N)	Number of positive detected (N)	Positive rate (%)
M	A	1.85	24	24	100
		1.54	24	24	100
		1.24	24	21	88
	C	1.85	24	24	100
		1.54	24	23	96
		1.24	24	19	79
	D	1.85	24	24	100
		1.54	24	23	96
		1.24	24	16	67
F		1.85	24	24	100
		1.54	24	23	96
		1.24	24	16	67
G		1.85	24	24	100
		1.54	24	23	96
		1.24	24	19	79
CRF01 AE		1.85	24	24	100
		1.54	24	23	96
		1.24	24	12	50
CRF01 AG		1.85	24	24	100
		1.54	24	24	100
		1.24	24	20	83
H		1.85	24	24	100
		1.54	24	23	96
		1.24	24	19	79
Group O		1.85	24	24	100
		1.54	24	23	96
		1.24	24	20	83
Group N		1.85	24	24	100
		1.54	24	23	96
		1.24	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 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/㎖ to 1.7 log_{10}IU/㎖ for HIV-1 Group M(subtype B) was prepared and three (3) dilution of each panel from 3.0 log_{10} IU/㎖ to 1.7 log_{10} IU/㎖ 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 to at least 8.00 Log_{10}IU/㎖, 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/㎖.

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

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(Log10 IU/㎖)	Linear range
A	y = 1.053x - 0.007	-0.18	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
B	y = 1.006x - 0.104	Not applicable	1.70 Log_{10}IU/㎖ to 8.00 Log_{10} IU/㎖
C	y = 1.090x - 0.181	-0.07	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
D	y = 1.092x - 0.214	-0.04	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
F	y = 1.034x - 0.088	-0.06	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
G	y = 1.109x - 0.237	-0.04	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
CRF AE	y = 1.080x - 0.175	-0.05	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
CRF AG	y = 1.087x - 0.133	-0.11	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖
H	y = 1.101x - 0.272	0.01	1.70 Log_{10}IU/㎖ to 3.00 Log_{10} IU/㎖

Table 8. LOQ of all HIV-1 subtypes analyzed

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

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 result 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

Table 10. The summary result 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

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 Log_{10} IU/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 substance	Concentration (ug/mℓ)	No.	Potential interfering substance	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 substance	Concentration	No.	Potential interfering substance	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/㎖.

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/㎖)	Test number	Detection rate (%)
2.00	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 log_{10} IU/㎖) and Negative HIV-1 free matrix, respectively.

Table 15. Cross contamination results

Run	Number of samples (detected/ tested)		Sample information(Log_{10} IU/㎖)	
	Positive	**Negative**	**8.00**	**Negative**
Run1	8/8	0/8	8.29	Not detected
Run2	8/8	0/8	8.29	Not detected
Run3	8/8	0/8	8.27	Not detected
Run4	8/8	0/8	8.25	Not detected
Run5	8/8	0/8	8.27	Not detected
Average			**8.28**	-
SD			**0.035**	-

Table 16. Summary of cross-contamination results (Between equipment)

Equipment	Number of samples (detected/ tested)		Sample information(Log_{10} IU/㎖)	
	Positive	**Negative**	**8.00**	**Negative**
Equipment 1	8/8	0/8	8.22	Not detected
Equipment 2	8/8	0/8	8.26	Not detected
Equipment 3	8/8	0/8	8.26	Not detected
Equipment 4	8/8	0/8	8.23	Not detected
Equipment 5	8/8	0/8	8.33	Not detected
Average			**8.26**	-
SD			**0.060**	-

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 a 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/㎖.

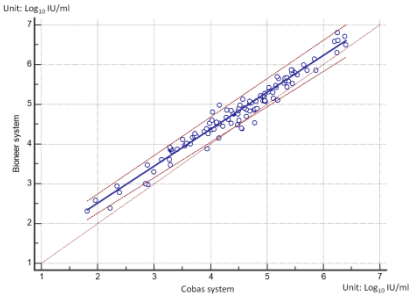


Fig. 77 Correlation with CE-IVD approved assay.

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**	
	σ_{within}	σ_{total}	S_{within}	S_{total}	S_{within}	S_{total}
100 IU/㎖	0,29	0,34	0,19	0,18	0,45	0,48
1,000 IU/㎖	0,09	0,13	0,06	0,07	0,14	0,20

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 ☞ 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** • Incorrect extraction or PCR kit use ☞ Make sure that you use proper kits for the intended tests. • The kit may have spoiled, due to bad storage or expiration. ☞ 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** • Invalid results. ☞ It must be tested with the new reagent
If the TAMRA (IPC) Fluorescence signal was not detected in particular wells.	• Inhibition of PCR ☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay from the sample pretreatment process which can reduce PCR inhibition. ☞ Make sure that you use the validated sample pretreatment method in accordance with the sample type. • Low elution volume due to insoluble material of samples ☞ 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. ☞ 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** • Re-use of reagents ☞ 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. See ***User's Guide*** **5. STORAGE CONDITION AND SHELF LIFE,** **7. General Precautions** • PCR Protocol error ☞ Review your reaction preparation procedure. Confirm the amount of SPC used in a single well. See ***User's Guide*** **8. PROTOCOL** • There may have been a pipetting error. ☞ Review the pipetting technique and calibration. • Invalid results. ☞ 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. ☞ Make sure that work space and instruments are decontaminated and repeat the assay. • The kit may have spoiled, due to bad storage or expiration. ☞ 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** • PCR Protocol error ☞ 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)). See ***User's Guide*** **8. PROTOCOL** • There may have been a pipetting error. ☞ Review the pipetting technique and calibration.

11. REFERENCES

Mackay IM. (2004) Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 10:190-212

Erali M, Hillyard DR. Evaluation of the ultrasensitive Roche Amplicor HIV-1 monitor assay for quantitation of human immunodeficiency virus type 1 RNA. J Clin Microbiol. 1999;37:792-5.

Fauci AS and Lane HC. Human immunodeficiency virus (HIV) disease: AIDS and related disorders. In Fauci AS, Braunwald E, et al. eds. Harrison's principles of internal medicine. 14th ed. New York: McGraw-Hill Companies, Inc., 1998:1791-856.

Hashida S. Hashinaka K, lihikawa S. hbikawa E. More reliable diagnosis of infection with human immunodeficiency virus type 1 (HIV-1) by detection of antibody IgGs to pol and gag proteins of HIV-1 and p24 antigen of HIV-1 in urine, saliva, and/or serum with highly sensitive and specific enzyme immunoassay (immune complex transfer enzyme immunoassay): a review. J Clin Lab Anal 1997;11: 267-86.

Hashida S. Hashinaka K, Nishikata I, Oka S. Shirnada K, Saito A, et al. Shortening of the window period in diagnosis of HIV-1 infection by simultaneous detection of p24 antigen and antibody IgG to p17 and reverse transcriptase in serum with ultrasensitive enzyme immunoassay. 1 Virol Methods 1996;62: 43-53.

Highbarger HC, Alvord WG, Jiang MK, Shah AS, Metcalf JA, Lane HC, Dewar RL. Comparison of the Quantiplex version 3.0 assay and a sensitized Amplicor monitor assay for measurement of human immunodeficiency virus type 1 RNA levels in plasma samples. J Clin Microbiol 1999;37:3612-4.

12. SYMBOLS

	Catalog number		Temperature limitation
	In vitro diagnostic medical device		Contains sufficient for test
	Manufacturer		Caution, consult accompanying documents
	Batch code		Expiration date
	Do not reuse		Consult instructions for use
	Warning for hazardous and irritation		Keep away from sunlight
CE 0197	Conformite Europeenne Mark	EC REP	Authorized representative in the European Community

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