USER'S GUIDE

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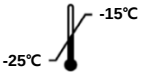
AccuPower®
HBV Quantitative PCR Kit



REF **HBV-1111**

IVD Quantitative test kit
Hepatitis B virus DNA

BIONEER
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AccuPower® HBV Quantitative PCR Kit

User's Guide

Version No.: 4.3 (2020-03-10)

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.

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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® HBV Quantitative PCR Kit is an *in vitro* diagnostic kit designed for quantification of HBV (Hepatitis B Virus) DNA in human EDTA-plasma samples or human serum through real-time polymerase chain reaction (PCR) using *ExiStation*™ Universal MDx system. *AccuPower*® HBV Quantitative PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers for monitoring of patient`s prognosis or treatment by measuring HBV viral load. The linearity of *AccuPower*® HBV Quantitative PCR Kit was 1.18 ~ 8.00 $Log_{10}IU/mℓ$ and the linearity of HBV was equal to linearity of each genotype (A,B,C,D,E,F,G and H). This kit is not intended to be used as a screening test for HBV infection in clinical samples including blood and blood products. It is not intended for initial clinical diagnosis of HBV infection, like an HBV screening.

2. INTRODUCTION

HBV is one of the smallest viruses known to infect humans, and belongs to the hepadnavirus family. It is a hepatotropic virus, and liver injury occurs through immune-mediated killing of infected liver cells.[1] The spectrum of disease and natural history of chronic HBV infection are diverse and variable, ranging from an inactive carrier state to progressive chronic hepatitis B (CHB), which may evolve to cirrhosis and hepatocellular carcinoma (HCC)[2] Eight genotypes of hepatitis B virus (A-H) are currently recognized, The genotypes show a distinct geographical distribution between and even within regions, and are proving to be an invaluable tool in tracing the molecular evolution and patterns and modes of spread of hepatitis B virus.

HBV is spread predominantly by percutaneous or mucosal exposure to infected blood and various body fluids, including saliva, menstrual, vaginal, and seminal fluids, which have all been implicated as vehicles of human transmission. Infection in adulthood leads to chronic hepatitis in less than 5% of cases. Transmission of the virus may also result from accidental inoculation of minute amounts of blood or fluid during medical, surgical and dental procedures, or from razors and similar objects contaminated with infected blood; use of inadequately sterilized syringes and needles; intravenous and percutaneous drug abuse; tattooing; body piercing; and acupuncture.[2]

The most common marker for detecting an HBV infection is characterized by the presence of antibodies (anti-HBs and anti-HBc) and HBeAg is used as a secondary marker to indicate active HBV replication associated with progressive liver disease. HBV DNA concentrations quantified by real-time polymerase chain reaction

(PCR) correlate with disease progression and are very useful to evaluate the efficacy of antiviral therapy. *AccuPower®* HBV Quantitative PCR Kit allow detection of HBV genotype A-H. 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 HBV VL assay systems.

3. FEATURES AND PRINCIPLE OF THE TEST

Real-time PCR involves the selective amplification of a dual target sequence (HBV surface antigen) while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. 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

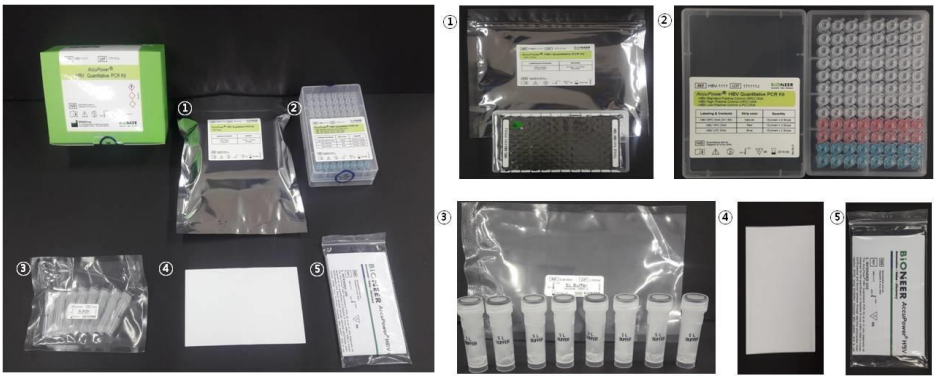


Fig. 1 Contents of *AccuPower*® HBV Quantitative PCR Kit

No.	Reagent	Unit	Quantity	Function
①	HBV PCR Premix	8-well/strip X 12 strips (96 tests) (in aluminum foil bag)	1 pack	NA amplification
②	HBV SPC[a] DNA 1 (S1) ; 2 X 10^2 copies/㎕	15 ㎕ / well (Natural 8-tube strip)	8 strips	Calibration
	HBV SPC[a] DNA 2 (S2) ; 2 X 10^3 copies/㎕			
	HBV SPC[a] DNA 3 (S3) ; 2 X 10^4 copies/㎕			
	HBV SPC[a] DNA 4 (S4) ; 2 X 10^5 copies/㎕			
	HBV SPC[a] DNA 5 (S5) ; 2 X 10^6 copies/㎕			
	HBV LPC[b] ; 2 X 10^2 copies/㎕	15 ㎕ / well (Blue 8-tube strip)	2 strips	Positive Control
	HBV HPC[c] ; 2 X 10^4 copies/㎕	15 ㎕ / well (Red 8-tube strip)	2 strips	
③	SL buffer	1800 ㎕ / tube (Clear 2㎖ screw tube)	8 tubes	Control loading buffer (for NTC[d], SPCs, PCs), Sample dilution
④	Quick Manual	-	1 ea	
⑤	Optical sealing film	-	1 ea	Sealing of PCR Premix
⑥	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. For detailed instructions, see 8. Protocol in this *User's Guide*.

5. STORAGE CONDITION AND SHELF LIFE

The *AccuPower*® HBV Quantitative 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 HBV premixed qPCR tube, the SPCs (HBV SPC (S1)-(S5)) and PCs (HPC/LPC) should be avoided, as this may reduce assay performance. If intermittent use of the kit and component (HBV premixed qPCR tube, the SPCs and PCs) is expected, HBV premixed qPCR tube are stable for up to 10 freeze/thaw cycles and SPCs (HBV SPC (S1)-(S5))/ PCs (HPC/LPC) are stable for up to 3 freeze/thaw cycles.

6. REQUIRED MATERIALS AND EQUIPMENT (NOT PROVIDED IN THE KIT)

System	Instrument	Reagent (Extraction)
ExiStaion™ **(A-2200)**	-*ExiPrep*™16 Dx (Cat. No. A-5050) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060)	- *ExiPrep*™ Dx Viral DNA Kit (K-4472) - *ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471)
ExiStaion™ **V4 (A-2200-N)**	-*ExiPrep*™16 Dx (Cat. No. A-5050) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block ver.4 (Cat. No. A-2060-1)	- *ExiPrep*™ Dx Viral DNA Kit (K-4472) - *ExiPrep*™ Dx Viral DNA/RNA Kit (K-4471)
ExiStaion™ **48 (A-2400)** ***ExiStaion***™ **48A (A-2410)**	- *ExiPrep*™48 Dx (Cat. No. A-5150) -*Exicycler*™ 96 Real-Time Quantitative Thermal Block ver.4 (Cat. No. A-2060-1) -*ExiLT* (Cat.No. A-7100)	- *ExiPrep*™ 48 Viral DNA/RNA Kit (K-4571)
Etc	- *ExiSpin*™ (Cat. No. A-7040)	N/A

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.

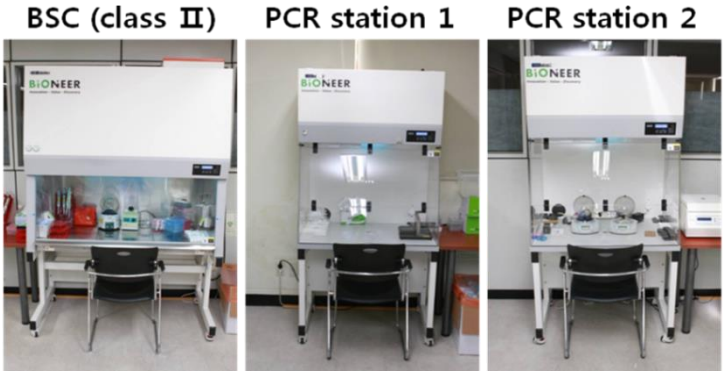
8. PROTOCOL

8.1 Laboratory equipment and environment

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

When handling clinical samples, all related works (i.e. de-capping, pipetting, capping of clinical samples and containers) **should be conducted within a negative pressure biosafety cabinet (class Ⅱ or Ⅲ).** Negative pressure biosafety cabinet sends air from the laboratory space outside. In other words, air flows inward. 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. Positive pressure biosafety cabinet is a workspace where filtered air flows outward, thus keeping a clean environment within the workspace.



Handling clinical specimens | Preparation of nucleic acid preparation kit contents | After the nucleic acid preparation, sealing and vortexing the PCR tubes.

Fig. 2 Biosafety Cabinet (BSC)

8.2 Specimen

All samples should be treated as potential biohazards. For the best results, we recommend DNA extracted from human EDTA-plasma samples or from human serum.

8.2.1 Specimen Collection

The *AccuPower*® HBV Quantitative PCR Kit is optimized for DNA extracted from human EDTA-plasma samples or from human serum. For EDTA-plasma collection or for human serum, 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 for up to 24 hours at 2°C to 30°C.

8.2.3 Specimen Storage

The isolated human EDTA-plasma or human serum can be stored up to 6 days at 2~8℃. For long period of storage, samples should be stored at following condition. Room temperature (25~30℃) for up to 1day or Temperature between 2 and 8℃ for up to 6 days, between -80 and -70℃ for 6 months.

8.2.4 Interfering Substances

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

8.3 Work Flow

The *AccuPower*® HBV Quantitative PCR Kit is designed for use with *ExiStation*™ Universal Molecular Diagnostic System.

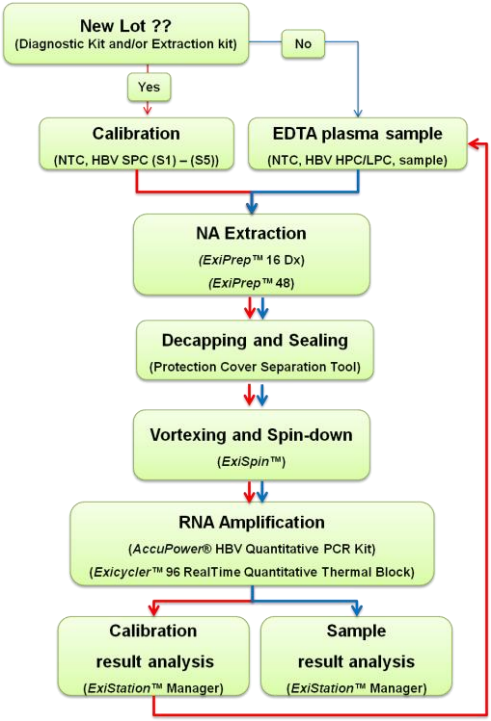


Fig. 3 Work flow

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

8.4 Part 1. Assigning test using *ExiStation*™ Manager program

1) Turn on the computer, which is pre-installed with *ExiStation*™ Manager Software.

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

Fig. 4 *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' image displayed on the LCD to initiate instrument startup.

Fig. 5 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).

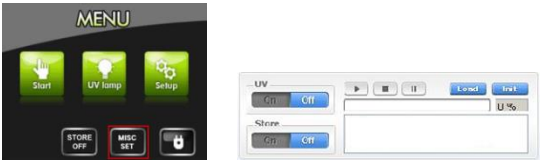
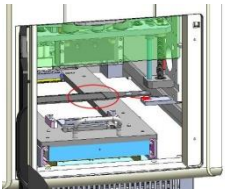
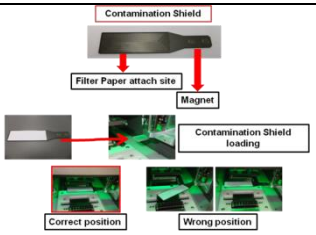


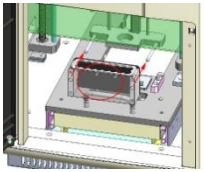
Fig. 6 LCD screen of *ExiPrep*™16 Dx and Load button of *ExiStation*™ Manager Software

5) 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.



Loading the Contamination Shield





Loading the Tip Protector

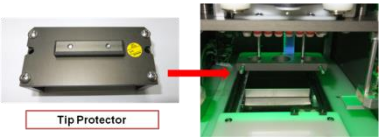


Fig. 7 Loading the Contamination Shield, Tip Protector

6) *ExiStation*™ Manager Software has six distinct parts.

Prep – control nucleic acid extraction (*ExiPrep*™16 Dx instrument),
Assign PCR – transfer sample information from 'Prep' to 'PCR' (*Exicycler*™ 96) and assign for PCR run
PCR – show real-time amplification conditions (*Exicycler*™ 96)
Result – when PCR is complete, present result, experiment and sample information
Configuration – software set-up information (accessible only by manufacturer)
Version – present software version

Fig. 8 Main screen of *ExiStation*™ Manager software

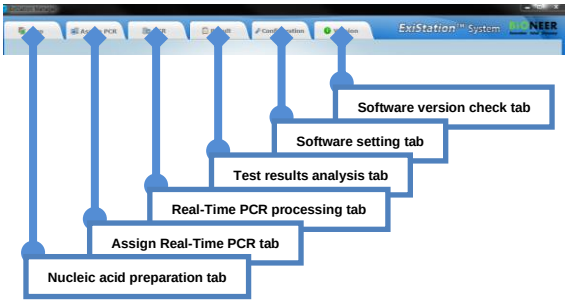


Fig. 9 Tab function of *ExiStation*™ Manager software

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

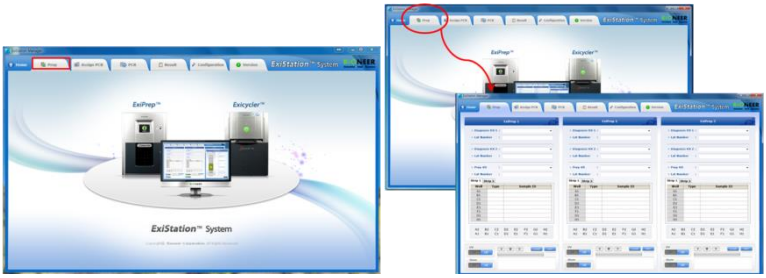


Fig. 10 Prep control panel consist of 5 panels

Prep control panel consist of 5 panels.

Instruments status panel – Status of *ExiPrep*™16 Dx

Kit selection panel –Select/enter the diagnostic kit, prep kit, and lot (or scanning the barcode of kits) information

Sample and control information panel – Enter control (NTC, PC, SPC) and sample (or scan the barcode of sample) information

Well information panel – Represent the well information with different color

***ExiPrep*™ 16 Dx control panel** – Control button of *ExiPrep*™ 16 Dx including UV controller, Store controller, Running controller, MISC set controller

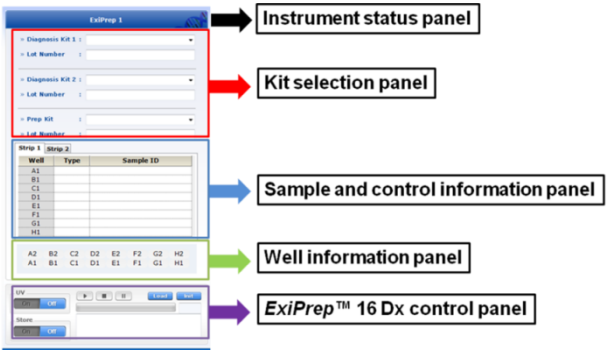


Fig. 11 Prep control panel of *ExiStation*™ Manager software

8) Click the pull-down arrow for 'Diagnosis Kit 1'. A popup window will appear. Select 'HBV-1111' from the pull-down menus.

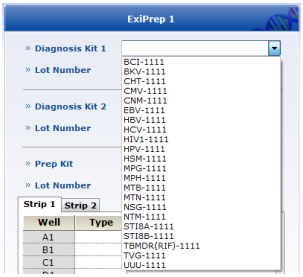


Fig. 12 Selection of Diagnostic kit

9) After selecting the 'Diagnosis Kit', a popup window will disappear. 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.

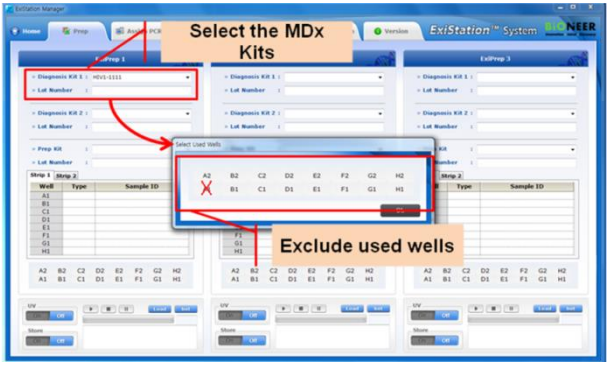


Fig. 13 'Prep' Pop-up window of *ExiStation*™ Manager software

10) 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.

11) 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 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.

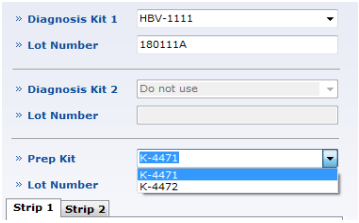


Fig. 14 Entering lot number

12) Click the 'Sample ID' column and enter sample information either using a barcode reader (optional) or type in manually.

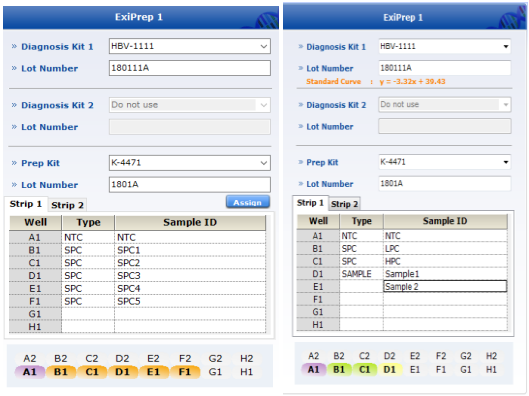


Fig. 15 Enter Sample ID(First assay/Repeated assay)

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

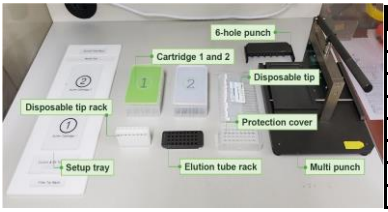
1) Bioneer recommend the using the BSC (Class II) and clean bench for *ExiStation*™ system operation.

2) Clean the surface (preferably a positive pressure BSC) 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 in order 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 preparation 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. 16 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 bottom of wells.

Fig. 17 Remove the lids

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

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

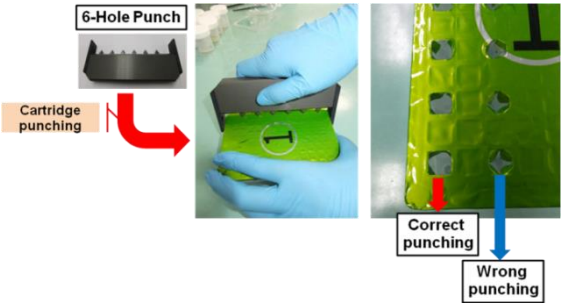


Fig. 18 Punch the film with the Hole Puncher

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

7) Place Buffer Cartridges on the setup tray.

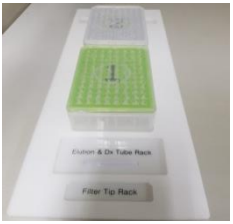


Fig. 19 Install buffer cartridge on the set-up tray

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. We recommend marking each strip of the diagnostic tubes with the corresponding column number.

⚠ You MUST make sure 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.

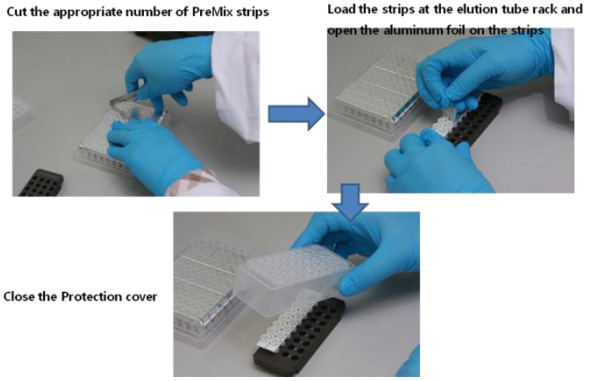


Fig. 20 Inserting the *AccuPower*® Diagnostic Kit tubes into Elution Tube Rack

9) 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.

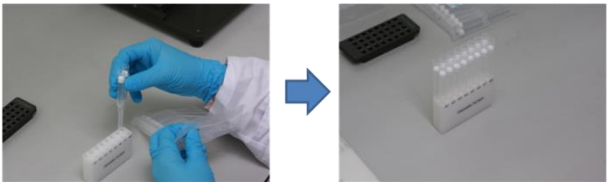


Fig. 21 Load the disposable filter tips at the disposable tip rack

11) Place the disposable tip rack on the setup tray

12) Place the waste tray on the setup tray.

Fig. 22 Load the Elution Tube Rack 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.

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

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 press the right-hand side of the cartridge firmly.

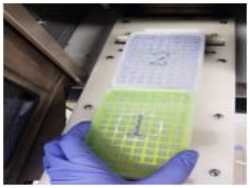


Fig. 23 Loading the Buffer Cartridge①

16) Place the Elution Tube Rack and Disposable Tip Rack on the base plate.
Note: Check the Protection Cover is properly secured on the Elution Tube Rack.

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.

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

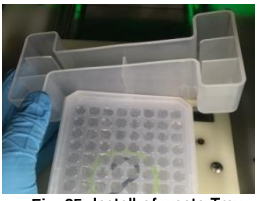


Fig. 25 Install of waste Tray

18) Prepare of 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 in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.

⚠Turn off the UV lamp when using the BSC.

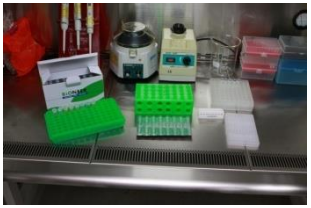


Fig. 26 Necessary components preparing for sample loading

19) Take out the DNA IPC Sample Loading Tubes from the packaging, mark it with sample name and insert them into the rack.

⚠Before using a Sample Loading Tube, bottom of Sample Loading Tube MUST BE check for Blue color (Dried IPC for DNA)

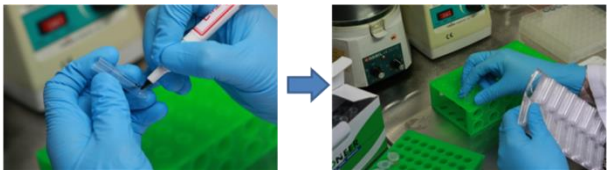


Fig. 27 Preparing Sample Loading Tube

20) Take the original clinical sample containers and controls (NTC and SPC) and pipette into the DNA IPC Sample Loading Tubes by following steps 21) ~ 23).

21) Add 400㎕ of SL buffer into a tube assigned as NTC. (supplied with the *AccuPower*® Diagnostic Kit)

22) Additionally add 395㎕ SL buffer and 5 ㎕ of SPC 1~5 into the appropriate SPC wells. (supplied with the *AccuPower*® Diagnostic Kit)

⚠ **When the assay is repeated with the same lot combination of Diagnostic kit and extraction kit, add 400 ㎕ of LPC (blue cap) and HPC (red cap) into each assigned well. calibration is Not needed this case.**

NTC: Add SL buffer 400 ㎕ for NTC well.
LPC: Add 395㎕ SL buffer and 5 ㎕ of **LPC (Blue) into LPC well.**
HPC: Add 395㎕ SL buffer and 5 ㎕ of **HPC (Red) into HPC well.**

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

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

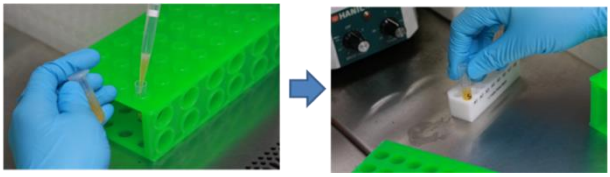


Fig. 28 Load clinical sample to Sample Loading Tube

24) Uncap clinical sample container and pipette 400 ㎕ sample into DNA IPC Sample Loading Tube. Move the DNA IPC Sample Loading Tube into Sample Tube Rack when it is filled with sample.

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

⚠If for any reason glove or tip contamination by sample is suspected, immediately exchange gloves or a tip to prevent contamination of samples.

26) Remove the waste tray on the base plate

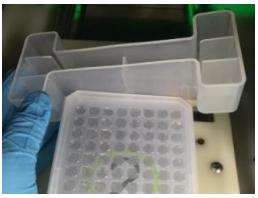


Fig. 29 Remove the waste tray

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

Fig. 30 Install of Sample Tube Rack

28) 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.

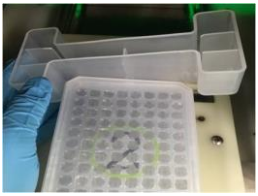


Fig. 31 Install of Waste Tray

29) All materials are loaded.

30) Remove the lids from Buffer Cartridges.

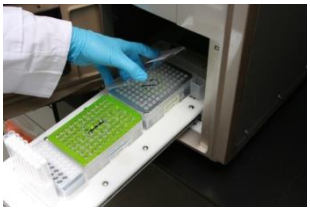


Fig. 32 Remove the lids

31) Check whether all accessories are loaded properly.

⚠ Make sure the tips, holes and tubes are all in alignment.

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

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

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

⚠ Nucleic acid extraction process takes 80~100 minutes according to the type of nucleic acid.

⚠ If any error messages appear during the extraction process. contact your local Bioneer`s distributor or headquarter for technical assistance.

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

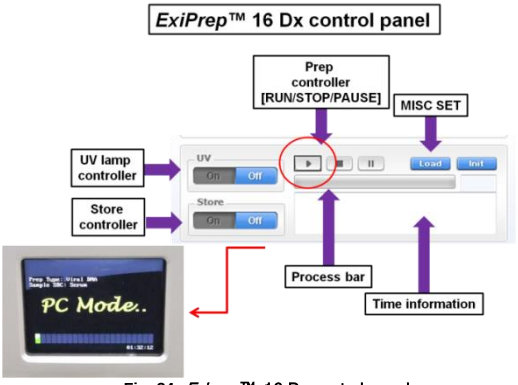


Fig. 34 *Exiprep*™ 16 Dx control panel

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

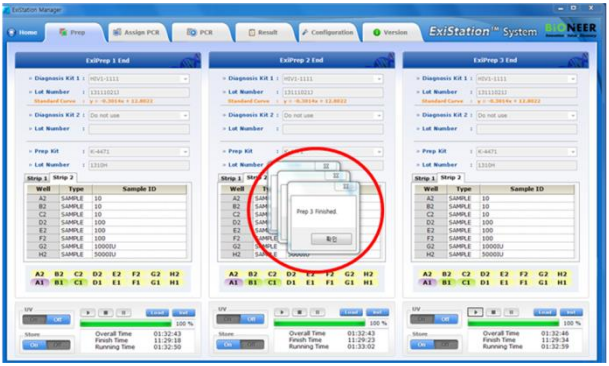


Fig. 35 Pop-up message for extraction finished

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, 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 Elution Tube Rack on Protection Cover Separation Tool, the lever must be facing left-hand side.

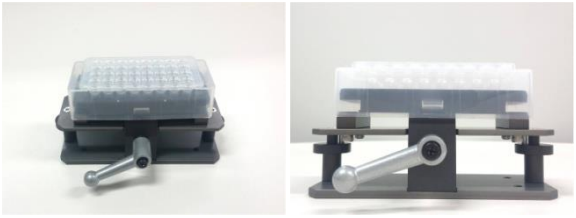


Fig. 37 Picture of Elution Tube Rack on top of Protection Cover Separation Tool

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.

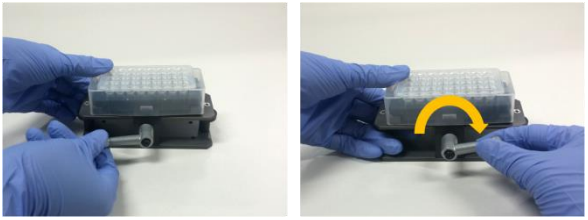


Fig. 38 Picture of lever rotation for fixing Elution Tube Rack to Protection Cover Separation Tool

③ Press down both sides of Separation Tool as shown in the picture 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 Separation Tool consecutively to prevent any liquid from splashing.

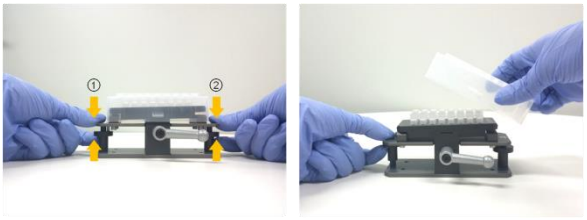


Fig. 39 Picture of pressing down each side of Separation Tool and removing Protection Cover from Separation Tool

5) Seal PCR Tube using Optical sealing film and then proceed to next step. For more information on 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).

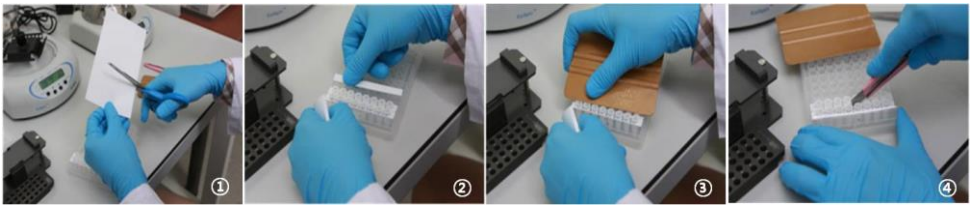


Fig. 40 Seal PCR premix strip

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.

⚠ MAKE SURE TO mark each diagnostic kit to prevent mix up.

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

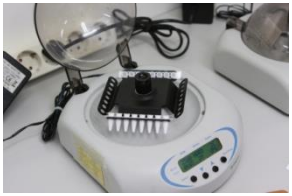


Fig. 41 Mix the PCR Premix Strip using *ExiSpin*™

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

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

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

9) Click '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.

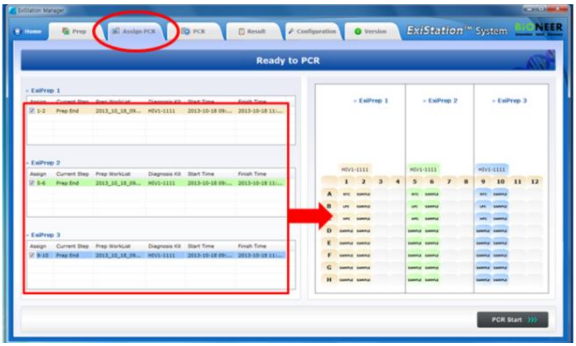


Fig. 43 'Assign PCR' tab – PCR Start

10) 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 complete, push the Door Switch for 2 seconds to close the door.

⚠ Make sure 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.

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

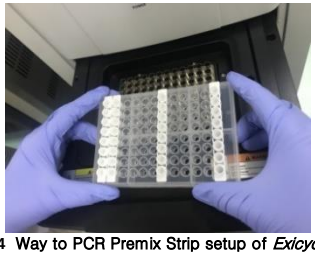


Fig. 44 Way to PCR Premix Strip setup of *Exicycler*™ 96

12) Select 'Assign PCR' tab and confirm 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 bottom right hand side of the window.
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'.**

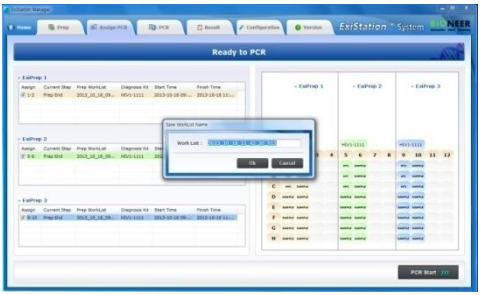


Fig. 45 Pop-up window of "Data name"

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

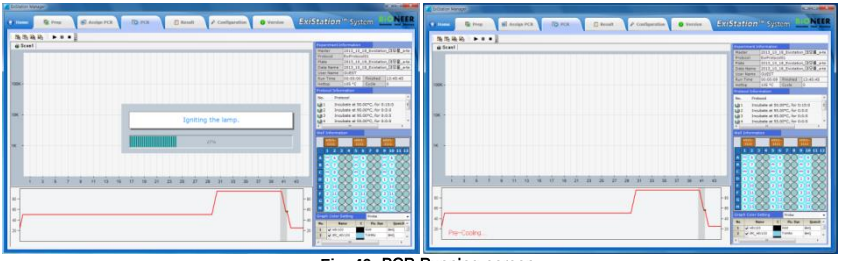


Fig. 46 PCR Running screen

14) 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 positive pressure BSC for later use.
>
> ⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.

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

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

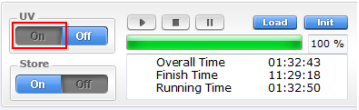


Fig. 47 *ExiPrep*™16 Dx control panel – UV

17) After the PCR run is finished, select 'Result' tab to check the results of each samples.

⚠ Click '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 procedure.

Fig. 48 Result analysis using *ExiStation*™ Manager software

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

8.7. Experimental procedure (*ExiStation*™ 48, *ExiStation*™ 48A)

8.7.1. Nucleic acid extraction – *ExiPrep*™ 48 Dx

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

8.7.1.1. Basic operating methods of *ExiStation*™ 48 Manager software for experiment

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

2) As it starts initialization by itself, the LCD screen will automatically appear.

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

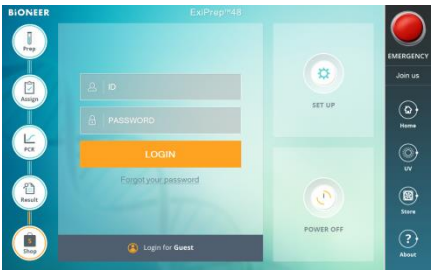


Fig. 49 Main screen of *ExiPrep*™ 48 Dx

4) Main screen consists of 5 icons.

Prep – Set-up and control nucleic acid extraction (*ExiLT*, *ExiPrep*™48 Dx)
Assign PCR – Extracted information can be displayed.
PCR – Monitoring extraction of Real-Time PCR (*Exicycler*™ 96)
Result – Show the results after executing PCR.
Shop – Links to homepage that can purchase related products.

5) Log in with the registered ID. When you log in as a guest, it is usually saved data in the folder specified. If you log in with your ID, you can specify a folder to store so that you can manage the resulting data more efficiently (optional).

6) Touch the Prep icon on the main screen. Screen will be changed as shown below 51. Enter Prep mode for nucleic acid extraction by touching the ExiStation.

Fig. 50 Initial screen of Prep tab

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

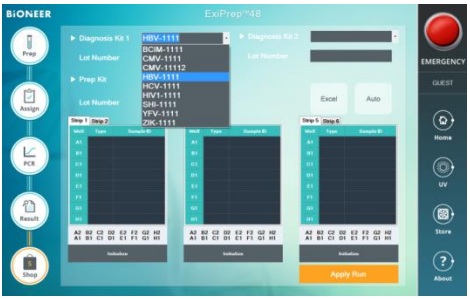


Fig. 51 Entering Diagnosis Kit information

8) Enter Lot number of the diagnosis kit.

9) Touch the pull-down arrow of Prep Kit. Show the diagnosis kit to use. Then enter Lot information of Prep Kit.

Fig. 52 Entering Kit information

10) As pop-up "Sample Type" message, select the sample to use

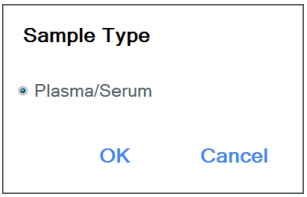


Fig. 53 Screen of 'Sample Type'

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

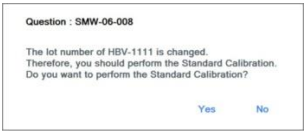


Fig. 54 Pop-up message for Standard Calibration process

12) 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. If there are not used well, click the "OK" button straight.

Fig. 55 Screen of 'Select Lane & Well'

13) NTC and SPC position is set automatically on remaining well, primary setting is NTC and each one of SPC 1~5. Repeat the same Lot number of Extract/Diagnosis kit's standard curve saves already. In this case LPC and HPC is set one well for each instead of SPC 1~5.

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

Fig. 56 Entering sample information

8.7.1.2. Nucleic acid extraction by *ExiPrep*™ 48 Dx

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

2) Clean the BSC and check that all necessary components for extraction and sample before nucleic acid extraction. Prepare extraction components within a positive pressure BSC. Recommend to perform at separated place referring to 8.1.1.

⚠	Clean the surface with 0.5% sodium hypochlorite and 70% ethanol or D.I water before and after use in 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 operation within positive pressure BSC–1.

Table 3. 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 the both Buffer Cartridges ① and ② within positive pressure BSC-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 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.

⚠ You MUST make sure 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. 57 Checking the position of diagnostic kit tubes in elution tube rack

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

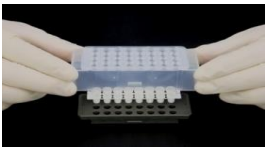
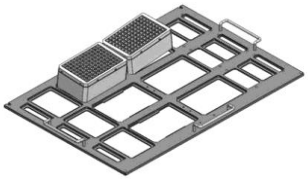


Fig. 58 Installing protection Cover

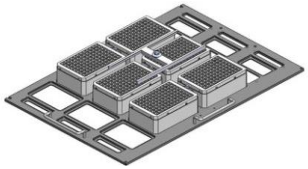
7) Open the door of the instrument (*ExiPrep*™ 48 Dx (A-5150)), remove set-up tray installed inside and place it on a flat experiment bench.

①



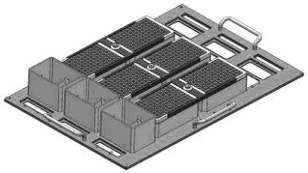
Install buffer cartridge ①, ②
to the sample quantity on the set-up tray.

②



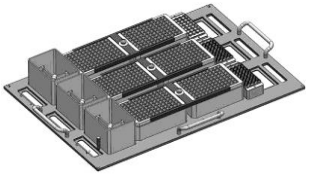
Install clamp on top of the buffer cartridge.
Clamps must be installed per lane.
And hold the clamp.

③



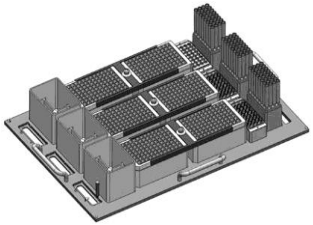
Install waste tray.

④



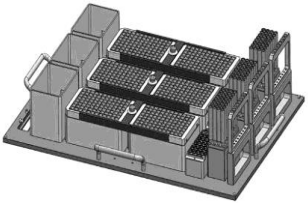
Install elution tube rack that installed PCR Premix Strip and protection cover to the set-up tray.

⑤



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

⑥



Install 8-hole punch.

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

9) Prepare of clinical samples in negative pressure BSC. 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 in 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 clean bench.

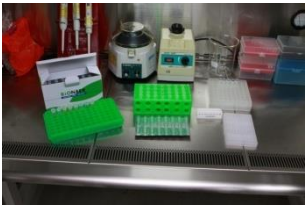


Fig. 59 Necessary components preparing for sample loading

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

⚠ Use sample loading tube with dried DNA IPC and check the blue color (DNA IPC) at the end of tube.

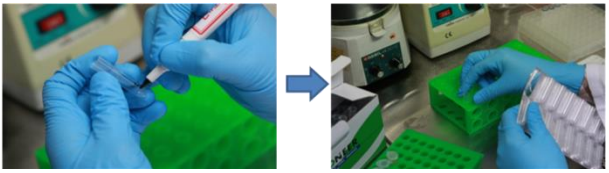


Fig. 60 Preparing Sample Loading Tube

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) Load SL buffer 400 ㎕(component of *AccuPower*® Diagnostic Kit) in NTC tube using pipette.

13) Load SL buffer 395 ㎕ in SPC tubes, add 5 ㎕ of SPC 1~5 in each tube.

> ⚠ If you have the pre-date of same lots of Diagnostic kit and Extraction kit, you may skip SPC calibration. 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 SL buffer **395 ㎕** in LPC tube and add LPC 5㎕(**blue tube,** component of *AccuPower*® Diagnostic Kit)
> **HPC** : Load SL buffer **395 ㎕** in HPC tube and add HPC 5 ㎕(**red tube,** 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 install, keep vertical direction during removal and installation of the rack to prevent 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 gloves or tip and so on are contaminated by clinical sample, remove the pollutant immediately. Then use new one.
> ⚠ Once the tube has been installed, push the fixing device to lock Sample Loading Tube's position

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

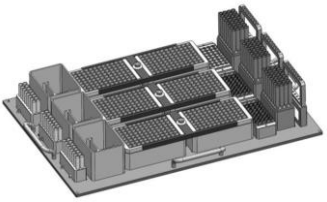


Fig. 61 Install of Sample Tube Rack

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

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

⚠ Check the each side, Left : Sample Tube Rack / Right : 8 hole punch, Then push the Setup tray into the instrument, carefully.

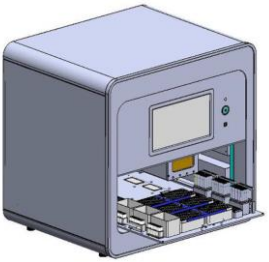


Fig. 62 Install of Setup tray

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

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

Fig. 63 Start extraction of nucleic acid though *ExiPrep*™ 48 software

8.7.2. Real-Time PCR using *Exicycler*™ 96

*** Please refer to user guide of *Exicycler*™ 96 and *ExiPrep*™ 48 software.**

1) Finish the extraction of nucleic acid, you will see 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, process after steps. The long delay can lead to degradation of nucleic acid, which may affect the result value.

2) Refer the 8.5.2 2)~6), Ready to PCR process after separate the PCR Premix Strip of Elution Tube Rack.

3) Click the "Assign" icon. The information list of finished nucleic acid extraction appears on the screen. Check the box what you want to PCR process. According to lane position of *ExiPrep*™ 48 Dx, decide the PCR well position.

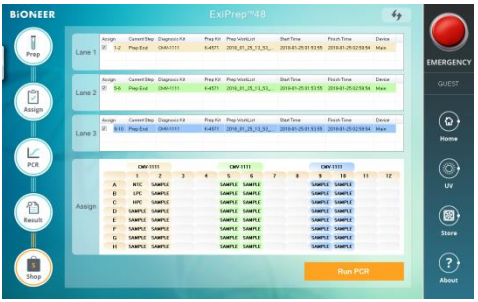


Fig. 64 Select the sample for PCR process

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

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

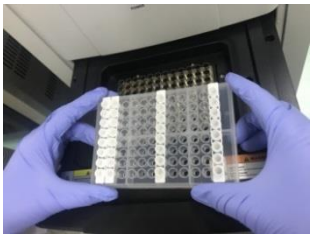


Fig. 65 Way to PCR Premix Strip setup of *Exicycler*™ 96

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

> ⚠ WorkList saves this way ; ExiStation™ 48 Manager program> SET UP > Data > WorkList

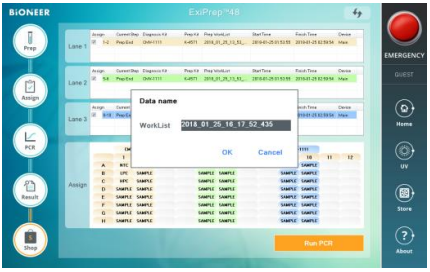


Fig. 66 Pop-up window of "Data name"

6) Complete 5) step, *Exicycler*™ 96 runs automatically.

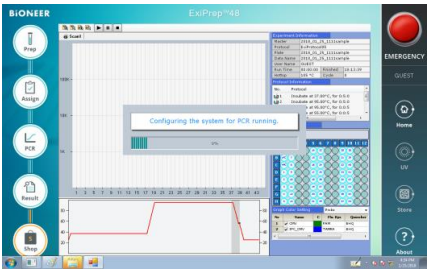


Fig. 67 PCR Running screen

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

- ⚠ Click "Analysis", an analysis program appears in a pop-up window and can confirm detail result.
- ⚠ After click the "Print" button(right of Analysis button), select the analysis result what you want to print can print as report.
- ⚠ Analysis result saves automatically on this folder
- ⚠ *ExiStation*™ 48 Manager program> SET UP > Data > WorkList > relevant data.

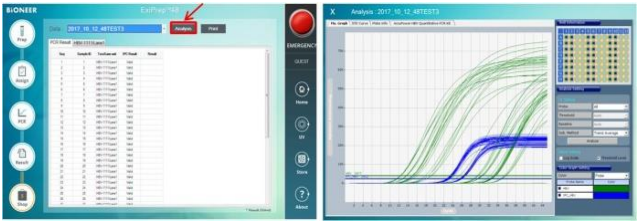


Fig. 68 Data analysis

8.8. Handling process of experimental waste

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 Filtertip from Buffer Catridge 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 positive pressure BSC for later use.
- ⚠ Cover the used Buffer Cartridges with the lids and discard them according to local safety regulations or internal laboratory procedure.

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

3) Push the Set-up tray in, shut the instrument door and initiate UV sterilization by clicking "UV ON" on the control panel.

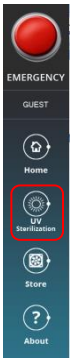


Fig. 69 *ExiPrep*™48Dx control panel – UV

4) After the PCR run is finished, select 'Result' tab to check the results of each samples.

⚠	DO NOT peel off an optical sealing firm from Diagnostic Kit. discard them according to local safety regulations or internal laboratory procedure

8.9 Data Analysis

(1) Calibration (HBV SPC (1) – (5))

For test with new Lot of diagnostic kit and/or extraction kit, calibration must be performed.

The test use 5 wells of SPC (HBV SPC (1)~(5)) to generate a standard curve. Additionally, the user can check for batch validity with *ExiStation™* manger software either by monitor or in a printed report. The batch is valid, if at least 3 SPCs are valid.

(2) Control (HBV LPC and HPC)

Every test is accompanied with control. The test use 2 wells of PCs (HPC, LPC) to confirm a validity of each test. The user can check the validity of test with *ExiStation*™ manger software either by 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 Ct value of HBV signal. If the assay is valid, HBV Ct will be 'undetermined' in NTC well and SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User's Guide section 10. Troubleshooting.

Specimen results are interpreted as follows:

Titer Result (IU/㎖*)	Interpretation
Not Detected	No Ct value (> 45 Ct) of HBV obtained. Reports are reported as "Not Detected".
<1.50E+01 IU/㎖	Calculated IU/㎖ are below the Limit of Quantification of the assay. Report results as "<1.50E+01".
≥ 1.50E+01 IU/㎖ and ≤ 1.00E+08 IU/㎖	Calculated results greater than or equal to 1.50E+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. Report results as "greater than 1.00E+08 IU/㎖". If quantitative results are desired, the original specimen should be diluted with HBV-negative human EDTA-plasma or human serum and the test repeated. Multiply the reported result by the dilution factor.

* IU/㎖ ; HBV DNA concentration in copy/㎖ X 0.7 IU/copy = HBV DNA in IU/㎖

8.10 Quality Control

(1) IPC (Internal Positive Control)

Every test tube contains an IPC to check PCR inhibition by the impurity or the mis-controlled thermal cycling in order to monitor the whole process. IPC is dried within Sample Loading tube (accessory for nucleic acid extraction, not provide). High concentrations of HBV DNA can lead to a reduced or absent fluorescence signal of the IPC due to PCR competition. The validity of IPC is determined by Ct value of IPC signal. If the Ct value is within specified range, it is valid. If the Ct value is out of specified range, it is invalid. The validity of SPC and NTC are determined by Ct value of HCV signal. If the assay is valid, HCV Ct will be 'undetermined' in NTC well and SPC Ct value will be within its specified range. If the control results are invalid, take measures according to User's Guide section 10. Troubleshooting.

The result of IPC determines the validity of the test and Ct value of HBV signal determines the HBV 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 CHARACTERISTICS

9.1 Analytical Characteristics

9.1.1 Limit of Detection (LoD)

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

AccuPower® HBV Quantitative PCR Kit detected HBV DNA with a detection rate of 95%, as determined by PROBIT, at a concentration of 6.02 IU/㎖ in EDTA-plasma and 10.47 IU/㎖ in serum.

Table 1. Detection rate of *AccuPower*® HBV Quantitative PCR Kit at each concentration in EDTA- plasma

Nominal concentration (Log_{10} IU/㎖)		Number of replicates tested (N)	Number of positives detected	Positive rate (%)
NTC	0	60	0	0
0.3	-0.51	60	11	18
0.63	-0.20	60	21	35
1.26	0.10	60	36	60
1.86	0.27	60	44	73
2.51	0.40	60	41	68
3.71	0.57	60	54	90
5	0.7	60	58	97
10	1	60	60	100

Table 2. Detection rate of *AccuPower*® HBV Quantitative PCR Kit at each concentration in Serum

Kit Lot	Nominal concentration (Log_{10} IU/㎖)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
NTC	0	72	0	0
0.63	-0.20	72	6	8
1.26	0.10	72	12	17
1.86	0.27	72	36	50
2.51	0.40	71	34	48
3.72	0.57	72	52	72

5.01	0.70	72	56	78
10.00	1	72	66	92
15.14	1.18	72	72	100

Table 3. Limit of Detection probit analysis in EDTA plasma

Concept	LoD	95% C.I	
IU/㎖	6.02	4.36	7.94
Log_{10} IU/㎖	0.78	0.65	0.90

Table 4. Limit of Detection probit analysis in Serum

Concept	LoD	95% C.I	
IU/㎖	10.47	8.30	13.18
Log_{10} IU/㎖	1.02	0.92	1.12

9.1.2 Verification of limit of detection for HBV genotype B – H

The verification of limit of detection of *AccuPower*® HBV Quantitative PCR Kit detection for genotype B–H was determined by analysis of 5 different dilutions levels of the World wide HBV DNA Performance Panel (Seracare code:WWHD301, USA) or 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques (PEI code 5086/08) in EDTA-plasma(Seracare, Milford, USA) and Serum (Millipore, Darmstadt, Germany) was used.

24-replicate was performed in each dilution and the study results demonstrate that the *Accupower*® HBV Quantitative PCR Kit can detect HBV DNA in EDTA plasma and Serum at a concentration as low as 0.78 and 1.00 Log_{10} IU/㎖, with a positivity rate greater than or equal to 95%.

Table 5. Detection rate of *AccuPower*® HBV Quantitative PCR Kit at each concentration in EDTA-plasma.

Genotype	Concentration (Log_{10} IU/㎖)	Number of replicates tested(N)	Number of positive detected	Positive rate (%)
B	1.30	24	24	100
	1.00	24	24	100
	0.88	24	24	100
	0.78	24	24	100
	0.70	24	23	96
C	1.30	24	24	100
	1.00	24	24	100

	0.88	24	24	100
	0.78	24	24	100
	0.70	24	23	96
D	1.30	24	24	100
	1.00	24	24	100
	0.88	24	24	100
	0.78	24	23	96
	0.70	24	23	96
E	1.30	24	24	100
	1.00	24	24	100
	0.88	24	24	100
	0.78	24	24	100
	0.70	24	24	100
F	1.30	24	24	100
	1.00	24	24	100
	0.88	24	24	100
	0.78	24	23	96
	0.70	24	24	100
G	1.30	24	24	100
	1.00	24	24	100
	0.88	24	24	100
	0.78	24	23	96
	0.70	24	20	88
H	1.30	24	24	100
	1.00	24	24	100
	0.88	24	24	100
	0.78	24	23	96
	0.70	24	24	100

Table 6. Detection rate of *AccuPower®* HBV Quantitative PCR Kit at each concentration in serum

Genotype	Concentration (Log_{10} IU/㎖)	Number of replicates tested(N)	Number of positive detected	Positive rate (%)
B	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
C	1.70	24	24	100
	1.40	24	24	100

	1.30	24	24	100
	1.18	24	24	100
	1.00	24	23	96
D	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
E	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
F	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	23	96
G	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	24	100
H	1.70	24	24	100
	1.40	24	24	100
	1.30	24	24	100
	1.18	24	24	100
	1.00	24	23	96

9.1.3 Linear range and Limit of Quantitation(LoQ)

Linearity and LoQ of HBV genotype A was performed with a dilution series of the WHO 3rd HBV International Standard panel (NIBSC code: 10/264, UK) for low titer members and HBV DNA plasmid, is test with *AccuPower*® HBV Quantitative PCR Kit.

The standard panel and HBV DNA plasmid were diluted in HBV negative human EDTA plasma and serum. The concentration of tested sample was from 0.88 Log_{10} IU/㎖ to 8 log_{10} IU/㎖ in EDTA plasma and 1.18 Log_{10} IU/㎖ to 8 log_{10} IU/㎖ in serum.

The evaluation of main LoQ and Linearity was performed with 3 different of *Accupower*® HBV Quantitative PCR Kit. Test was performed with each concentration 4 replicates and 1 runs per day and on 5 different days, on 3 different *ExiStation*™ system instruments, resulting in 60 overall data points per dilutions.

Linear range claim for *AccuPower*® HBV Quantitative PCR Kit was from 1.18 HBV DNA Log_{10} IU/㎖ to at least 8.00 HBV DNA Log_{10} IU/㎖, with maximum deviation between the observed mean Log_{10} titer and the best fitted 1st-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 1.18 to 8.00 Log_{10} IU/㎖.
At a concentration of 1.18 Log_{10} IU/㎖, it was satisfied the total analytical error (TE) value within 1.00 Log_{10} IU/㎖. Therefore, the claimed LOQ for the *AccuPower*® HBV Quantitative PCR Kit considering all HBV genotype is 1.18 Log_{10} IU/㎖.

Table 7. Linear equation results of HBV genotype in EDTA-plasma

HBV genotype	Linear equation in genotype linearity study	Maximum difference between HBV genotype A and corresponding HBV genotype (Log_{10} IU/㎖)	Linear range
A	y = 1.0749x – 0.2416	N/A	0.88 Log_{10} IU/㎖ to 8.00 Log_{10} IU/㎖
B	y = 1.0675x – 0.2507	0.07	0.88 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
C	y = 1.0425x – 0.165	0.18	0.88 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
D	y = 1.0499x – 0.2189	0.18	0.88 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
E	y = 1.0403x – 0.0075	−0.19	0.88 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
F	y = 1.0268x – 0.0266	0.17	0.88 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
G	y = 1.0542x – 0.1402	−0.08	0.88 Log_{10} IU/㎖ to 2.00 Log_{10} IU/㎖
H	y = 1.0834x – 0.1357	−0.17	0.88 Log_{10} IU/㎖ to 2.00 Log_{10} IU/㎖

Table 8. Linear equation results of HBV genotype in serum

HBV genotype	Linear equation in genotype linearity study	Maximum difference between HBV genotype A and corresponding HBV genotype (Log_{10} IU/㎖)	Linear range
A	y = 1.058x – 0.3768	N/A	1.18 Log_{10} IU/㎖ to 8.00 Log_{10} IU/㎖
B	y = 1.0733x – 0.3118	−0.19	1.18 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
C	y = 1.0426x – 0.2169	−0.14	1.18 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
D	y = 1.0206x – 0.1703	−0.16	1.18 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
E	y = 1.0615x – 0.2368	−0.17	1.18 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖
F	y = 1.0588x – 0.229	−0.15	1.18 Log_{10} IU/㎖ to 4.00 Log_{10} IU/㎖

G	y = 1.036x − 0.2114	−0.14	1.18 Log_{10} IU/㎖ to 2.00 Log_{10} IU/㎖
H	y = 1.065x − 0.2915	−0.14	1.18 Log_{10} IU/㎖ to 2.00 Log_{10} IU/㎖

Table 9. LOQ results of HBV genotypes in EDTA-plasma

HBV Genotype	Nominal concentration (Log_{10} IU/ml)	N	Average Measured Concentration (Log_{10} IU/ml)	IBiasI (Log_{10} IU/ml)	SD (Log_{10} IU/ml)	TE = |Bias| +2 x SD (Log_{10} IU/ml)	SQRT[2]x2 x SD (Log_{10} IU/ml)
A	1.18	60	0.97	0.21	0.20	0.61	0.56
B	1.18	30	0.99	0.19	0.16	0.51	0.46
C	1.18	30	0.92	0.26	0.28	0.81	0.79
D	1.18	30	1.02	0.16	0.18	0.52	0.51
E	1.18	30	1.09	0.09	0.17	0.43	0.49
F	1.18	30	1.26	0.08	0.21	0.50	0.59
G	1.18	30	1.04	0.14	0.15	0.44	0.43
H	1.18	30	1.00	0.18	0.22	0.61	0.61

Table 10. LOQ results of HBV genotypes in serum

HBV Genotype	Nominal concentration (Log_{10} IU/ml)	N	Average Measured Concentration (Log_{10} IU/ml)	IBiasI (Log_{10} IU/ml)	SD (Log_{10} IU/ml)	TE = |Bias| +2 x SD (Log_{10} IU/ml)	SQRT[2]x2 x SD (Log_{10} IU/ml)
A	1.18	60	0.73	0.45	0.25	0.94	0.69
B	1.18	30	0.94	0.24	0.29	0.82	0.83
C	1.18	30	1.09	0.09	0.22	0.53	0.62
D	1.18	30	0.98	0.20	0.28	0.76	0.79
E	1.18	30	1.08	0.27	0.31	0.89	0.88
F	1.18	30	0.97	0.20	0.31	0.83	0.88
G	1.18	30	1.04	0.13	0.15	0.44	0.43
H	1.18	30	1.07	0.11	0.28	0.66	0.78

9.1.4 Precision

Precision claim for *AccuPower*® HBV Quantitative PCR Kit was determined by analysis of international standard panel(3rd WHO International Standard) and HBV plasmid (Bioneer corp.). 3 dilution levels were tested in 80 replicates for each level across three lots of *AccuPower*® HBV Quantitative PCR Kit using *ExiStation*™ system for 25 days in EDTA-plasma and in serum(for repeatability).

The between-Lot experiment was conducted in 3 lots, 2 replicates per samples, and 2 runs per day during 5 days. The between-operator and between-instrument experiment was conducted in 1 lots, 2 replicates per samples, and 2 runs per days on during 10 days. The between-site experiment was conducted in 1 lot, 2 replicates per samples, and 2 runs per day on during 10 days.(for reproducibility).

Table 11.The summary result of repeatability in EDTA-plasma

Nominal Concentration (Log_{10} IU/㎖)	Assigned Concentration (Log_{10} IU/㎖)	No. of Valid tests	Within-Run(S_r)	Between-Run(S_{rr})	Between-Day(S_{dd})	Total precision(S_T)
6.00	6.44	80	0.09	0.04	0.06	0.12
3.33	3.46	80	0.10	0.03	0.02	0.11
1.26	1.23	80	0.16	0.10	0.10	0.21

Table 12.The summary result of repeatability in serum

Nominal Concentration (Log_{10} IU/㎖)	Assigned Concentration (Log_{10} IU/㎖)	No. of Valid tests	Within-Run(S_r)	Between-Run(S_{rr})	Between-Day(S_{dd})	Total precision(S_T)
6.00	5.65	80	0.15	0.13	0.07	0.21
3.33	3.19	80	0.10	0.04	0.03	0.11
1.65	1.33	80	0.18	0.10	0.13	0.24

Table 13.The result of reproducibility in EDTA-plasma

Plasma			Standard Deviation			
Nominal Concentration (Log_{10} IU/㎖)	Assigned Concentration (Log_{10} IU/㎖)	No. of Valid Tests	Between-Lot	Between-Site	Between-Operator	Between-Instrument
6.00	6.43	200	0.11	0.13	0.10	0.08
3.33	3.47	200	0.09	0.12	0.10	0.10
1.26	1.26	200	0.20	0.21	0.22	0.22

Table 14.The result of reproducibility in serum

Serum		Standard Deviation				
Nominal Concentration (Log_{10} IU/㎖)	Assigned Concentration (Log_{10} IU/㎖)	No. of Valid Tests	Between-Lot	Between-Site	Between-Operator	Between-Instrument
6.00	5.66	200	0.21	0.18	0.17	0.19
3.33	3.21	200	0.10	0.13	0.09	0.09
1.65	1.34	200	0.22	0.26	0.21	0.26

9.1.5 Interfering substances

Interfering effects by twenty-two (22) exogenous substances (included anti-viral substance) and by seven (7) endogenous substances was tested for interfering of *AccuPower*® HBV Quantitative PCR kit.

Potentially interfering endogenous and exogenous substances were spiked into EDTA plasma and into serum in the absence or presence of 2 (3.33, 1.26 Log10 IU/㎖ in Plasma and 3.33, 1.65 Log10 IU/㎖ in Serum) concentration of HBV and were compared to control EDTA plasma and serum samples containing no spiked interfering substance. Each concentration level for each interfering substance was tested in three (3) and eight(8) replicates.

All tested interfering substance concentrations was shown no influence on the performance of the *AccuPower*® HBV Quantitative PCR Kit at the HBV concentrations.

Table 15. Interference- Exogeonus Interfering Substances

No.	Potential interfering substance	Concentration
1	Raltegravir	25.8 mg/L
2	Entecavir	164ng/ml
3	Lamivudine	44.8ug/ml
4	Telbivudine	74ug/ml
5	Rifampicin	312mg/L
6	Trimethoprim	2760umol/ml
7	Isoniazid	5.84mmol/ml
8	Rifabutin	9.2mg/ml
9	Adefovir dipivoxil	368ng/L
10	Sulfamethoxazole	31.6mmol/L
11	Pyrazinamide	576ug/ml

12	Nevirapine	40ug/ml
13	Ritonavir	224ug/L
14	Ribavirin	54.96ug/L
15	Abacavir	60ug/ml
16	Tenofovir	6ug/ml
17	Zidovudine	45.8ug/ml
18	Efavirenz	81.4ug/ml
19	Saquinavir	104.16ug/ml
20	Nelfinavir	40ug/ml
21	Valganciclovir	113ug/ml
22	Amprenavir	153.2ug/ml

Table 16. Interference- Endogenous Interfering Substances

No.	Potential interfering substance	Concentration
1	EDTA	540mg/dL
2	Citrate	0.372M/ml
3	Heparin	600U/ml
4	Hemoglobin	40mg/ml
5	Cholesterol	200mg/ml
6	Albumin	10g/dL
7	Bilirubin	0.25mg/ml

9.1.6 Cross reactivity

The following viruses and Bacteria were tested for cross-reactivity of *AccuPower*® HBV Quantitative PCR Kit. Samples were prepared by diluting organisms either in HBV negative EDTA-plasma and in HBV negative serum or in HBV panel spiked EDTA-plasma and in HBV spiked serum and were tested in eight(8) or three(3) replicates.

negative HBV EDTA plasma and serum samples for negative were shown negative and HBV positive specimens spiked in cross-reactivity organisms were shown to detect within ±0.44 Log_{10} IU/㎖ in EDTA-plasma and ±0.65 Log_{10} IU/㎖ in serum

Table 17. List of potential cross reactivity organism

Viruses		Bacteria
Hepatitis A virus	Zika Virus	*Mycobacterium gordonae*
HIV-1	Human herpesvirus 6B	*Chlamydia trachomatis*
Hepatitis C virus	Human herpesvirus 8	*Neisseria gonorrhoeae*
Epstein-Barr Virus	HIV-2	*Staphylococcus aureus*
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		

9.1.7 Whole system failure

The Whole System Failure rate was tested with one-hundred twenty (120) replicates using *AccuPower*® HBV Quantitative PCR Kit. Positive results were obtained 100% detection of one-hundred twenty (120) replicates; overall, a system success rate was shown 100% in the *AccuPower*® HBV Quantitative PCR Kit in plasma and serum respectively.

Matrix	Concentration (Log_{10} IU/㎖)	Test number	Detection rate(%)
Plasma	1.26	120	100%
Serum	1.51	120	100%

9.1.8 Cross contamination

Cross-contamination test was performed using HBV diagnostic kit according to the CTS guideline. High positive and negative were tested at 7.00 Log_{10} IU/㎖ concentration and Negative HBV free matrix, respectively.

The *AccuPower*® HBV Quantitative PCR Kit showed no evidence of cross-contamination when tested with eighty (80) HBV positive samples (7.00 Log_{10} IU/㎖) and negative samples, which were loaded in an alternating order. Ten (10) extraction and ten (10) PCR runs were performed in total.

Run	Number of samples (detected/ tested)		Sample information(Log10 IU/㎖)		Pass/fail
	Positive	Negative	7.00	Negative	
Run1	8/8	0/8	6.79	Not detected	Pass
Run2	8/8	0/8	6.85	Not detected	Pass
Run3	8/8	0/8	6.70	Not detected	Pass
Run4	8/8	0/8	6.79	Not detected	Pass
Run5	8/8	0/8	6.80	Not detected	Pass
Run6	8/8	0/8	6.68	Not detected	Pass
Run7	8/8	0/8	6.70	Not detected	Pass
Run8	8/8	0/8	6.78	Not detected	Pass
Run9	8/8	0/8	6.59	Not detected	Pass
Run10	8/8	0/8	6.74	Not detected	Pass
Average			6.74	–	–
SD			0.12	–	–

9.1.9 Matrix Equivalency

A total of thirty (30) paired samples (serum and EDTA plasma samples) that was collected from HBV positive patients and HBV DNA positive were used for matrix equivalency with *AccuPower*® HBV Quantitative PCR Kit.

The slope of deming regression was shown a 1.0034 (95% confidence interval [0.970 to 1.036]) with an intercept of 0.2458 (95% confidence interval [0.131 to 0.360]).

Log10 IU/㎖ titer difference (mean log titer serum – mean log titer EDTA plasma) value of thirty(30) paired samples was shown within 0.5 log10 IU/㎖. as mean difference value was shown −0.26 Log10 IU/㎖(95% CI: 0.19 and 0.31), there were not significantly different between serum and EDTA-plasma.

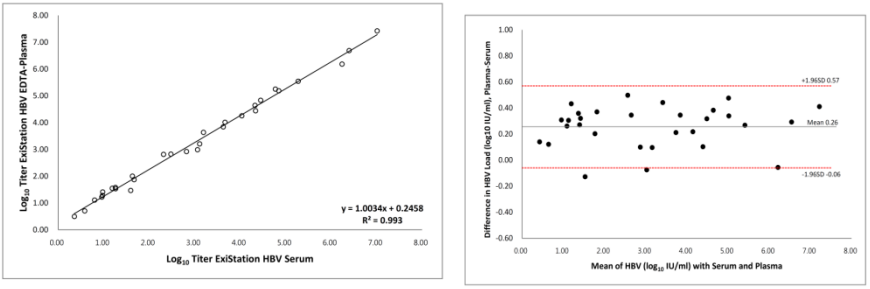


Fig. 70 PCR Running screen

Key Statistics		Deming Regression Statistics Y=Slope * X + Intercept	
correlation coefficient (R)	0.9965	Slope	1.0034(0.970 to 1.036)
coefficient of determination (R^2)	0.993	Intercept	0.2458 (0.131 to 0.360)
Bias Mean	0.26(0.19 to 0.31)	Std.Err of Estimate	0.162
Bias Std Dev.	0.16	Points (Plotted/Total)	30/30
Mean difference±2SD	−0.06 to 0.57	Outliers	0

9.2 Diagnostic Performance Characteristics

9.2.1 Diagnostic Sensitivity & Specificity

Total two- hundred- forty -four (244) clinical samples, which were confirmed with other HBV quantitative assay, were used for diagnostic sensitivity & specificity test.

The percent agreement between the test device under evaluation and the comparison device was calculated as follows:

		Reference system		
		Positive	Negative	Total
ExiStation™ system	Positive	133	0	133
	Negative	0	111	111
	Total	133	111	244

Diagnostic Sensitivity (Percent positive agreement) = **100 % (95% C.I 97.19 – 100)**

Diagnostic Specificity (Percent negative agreement) = **100 % (95% C.I 96.65 – 100)**

9.2.2 Correlation

Total one-hundred thirty-three (133) HBV positive EDTA plasma clinical samples were compared with CE-IVD approved HBV NAT assay.

The scatter plot for two assays is shown in correlation results graph, with the linear equation y = −0.529 + 1.048 X and correlation coefficient R^2 = 0.935, which indicated a significant correlation between the two assays.

The Bland Altman plot analysis was used to assess the difference between the positive results obtained with the two assays (Bland Altman graph). The results showed that 95.49% of positive data were within the 95% acceptable range (−0.44 to 1.16), the average difference value was 0.36.

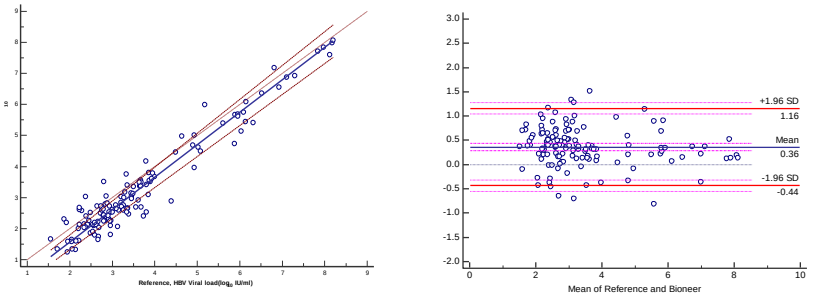


Fig. 71 Correlation with CE-IVD approved assay

9.2.2 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 HBV international standard panel that was tested with one lot of *AccuPower*® HBV Quantitative PCR Kit according to CLSI EP15-A. Two replicates of each dilution per day were 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*® HBV Quantitative PCR Kit assay was verified to be consistent with the manufacturer`s claim.

Table 18. summary results of User`s precision verification.

Concentration	Analytical performance Precision value		Verification performance Precision value		Verification performance verification value	
	σ_{within}	σ_{total}	S_{within}	S_{total}	S_{within}	S_{total}
1.26 log_{10} IU/㎖	0.16	0.21	0.16	0.14	0.25	0.33
3.33 log_{10} IU/㎖	0.10	0.11	0.10	0.09	0.16	0.16

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 AND SHELF LIFE • 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 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 AND SHELF LIFE 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 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.

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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

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