WHO Emergency Use Assessment and Listing for Zika IVDs
PUBLIC REPORT

Product: AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit
EUAL Number: EAZ 0006-004-00

Emergency Use Assessment and Listing of In Vitro Diagnostics Procedure

WHO has developed an Emergency Use Assessment and Listing (EUAL) procedure to expedite the availability of in vitro diagnostics (IVDs) needed in public health emergency situations. This EUAL procedure will generate WHO recommendations in order to provide advice to procurement agencies and Member States on the acceptability of a specific IVD in the context of a public health emergency, based on a minimum set of available quality, safety, and performance data and an agreed plan for further evaluation.

The EUAL procedure is comprised of three components that aim to assess the safety, quality and performance of the IVD:

- a review of the manufacturer's quality management system documentation;
- a review of the documentary evidence of safety and performance; and
- an independent performance evaluation

AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit with product code **ZIK-1111** manufactured by **Bioneer Corporation,** 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon 34302, South Korea, CE marked regulatory version, was listed as eligible for WHO procurement on **28 October 2016**.

The AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is an in vitro diagnostic kit designed for the qualitative detection of ZIKA, Dengue and, Chikungunya Virus RNA in human samples such as serum, plasma and urine (Only for ZIKV) samples through real-time reverse transcription polymerase chain reaction (RT-PCR).

Real-time PCR involves the selective amplification of a target sequence 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 labelling 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.

Content of the kit ZIK-1111 for use with BIONEER's ExiStation™ Universal Molecular Diagnostic System

Reagents	Unit	Quantity (96 test kit)
ZIKV Premix	1 Aluminum Foil Bag	8 tubes x 12 strips (96 tubes)
Positive Control RNA	15 µl / tube (Natural 8-tube strip)	8 tubes x 2 strips (16 tubes)
IPC RNA (Internal Positive Control)	15 µl / tube (Yellow 8-tube strip)	8 tubes x 2 strips (16 tubes)
DEPC-DW (No Template Control)	15 µl / tube (Purple 8-tube strip)	8 tubes x 2 strips (16 tubes)
DEPC-DW	1800 µl / tube	4 tubes
SL Buffer	1800 µl / tube	8 tubes
Optical Sealing Film	-	1
Quick Manual	-	1

Materials required but not provided

Material	Product name/description
Real-Time PCR instrumentation required for kit ZIK-1111	BIONEER's • ExiStation™ Universal Molecular Diagnostic System (Cat. No. A-2200): (Integrated platform of Real-Time PCR instrument (Exicycler™ 96) and nucleic acid extraction Instrument (ExiPrep™ 16 Dx)) • Exicycler™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060)
Reagent requirements for kit ZIK-1111	• ExiPrep™ 16 Dx (Cat. No. A-5050) • ExiPrep™ Dx Viral RNA Kit (Cat. No. K-4473) • ExiSpin™ (Cat. No. A-7040)
Materials requirements for kit ZIK-1111	• Disposable powder-free gloves • Appropriate volume pipette set • Sterilized pipette tips with filters • 1.5 ml micro tubes or 15 ml conical tubes

Storage: The test kit should be stored between -25°C and -15°C.

Shelf-life upon manufacture: 12 months

Precaution: For diagnostic quality control, refer to *Chapter 11, Recommendation for Maintenance of the laboratory environment and instrument* of the instructions for use (IFU).

WHO EUAL Assessment

Bioneer Corporation submitted an expression of interest for WHO emergency quality assessment of AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit on 31 March 2016.

Review of quality management documentation
To establish the eligibility for WHO procurement, Bioneer Corporation was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation, it was established that sufficient information was provided by Bioneer Corporation to fulfil the requirements described in the "Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO".

Quality management documentation for Emergency Use Assessment and Listing conclusion:
Acceptable

Product dossier assessment
Bioneer Corporation submitted documentation in support of safety and performance for AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit as per the "Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO"[1]. The information submitted in the product application was reviewed by WHO staff and external experts (reviewers) appointed by WHO. The findings of the reviews were reported in accordance with "Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting Zika Virus Nucleic Acid or Antigen" (WHO document number WHO PQDx_0240 v 2).

Safety and performance documentation for Emergency Use Assessment and Listing conclusion:
Acceptable

Laboratory evaluation
Analytical testing
The limit of detection of the assay was evaluated independently by the Paul Ehrlich Institut, Langen, Germany.

[1] Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO.

Testing was conducted using a material prepared as a candidate international standard (IS) developed by the Paul-Ehrlich-Institut (PEI), Langen, Germany on behalf of WHO. The candidate international standard has been assigned a potency of 50,000,000 IU/ml based on the results of an international collaborative study. The results of the study will be put forward to the Expert Committee on Biological Standardization (ECBS) for adoption in October 2016.

Reference material was reconstituted in 0.5 ml of nuclease-free water as recommended.

The Zika virus RNA extraction was performed using the ExiPrep™ Dx Viral RNA Kit on the ExiStation™ Universal Molecular Diagnostic System. Four hundred µL of specimen was used for each extraction. The entire volume of recovered RNA is then used in the subsequent amplification/detection reaction also using the ExiStation™ Universal Molecular Diagnostic System which comprises the ExiPrep™ 16 Dx platform for the extraction of nucleic acids as well as the ExiSpin™ and the Exicycler™ 96 Real-Time Quantitative Thermal Block.

A total of three runs were used to determine the assay's LoD:

- An initial run was conducted using 5 replicates of half log10 dilution steps between 6.0 log10 and -8.5 log10 IU/ml.
- A second run was conducted using 5 replicates of half log10 dilution steps between 5.5 log10 and -8.0 log10 IU/ml. Two additional replicates were used for the -6.5 log10 dilution.
- A third run was conducted using 5 replicates of half log10 dilution steps between 5.5 log10 and -8.0 log10 IU/ml.

The analytical sensitivity (95% limit of detection [LoD]) of **AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit** was determined by Probit analysis in plasma specimens to be 81.3 IU/ml, with the 95% confidence interval (CI) ranging from 34.9 to 189.6 IU/ml. Additional testing to determine the analytical sensitivity in urine specimens is ongoing. This report will be amended as soon as data becomes available.

Table 1. 95% LoD and confidence interval (CI)

	Plasma
95% LoD (CI) IU/ml	81.3 IU/mL (34.9-189.6)

Clinical Testing:
Clinical testing is scheduled. Results will be made available shortly.

Laboratory evaluation for Emergency Use Assessment and Listing conclusion:
Acceptable contingent on clinical evaluation results

WHO Emergency Use Assessment and Listing Decision

Based on the review of the manufacturer's submitted data, as well as data generated from the limited laboratory evaluation AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is eligible for WHO procurement.

Post market surveillance to monitor the performance of AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is highly recommended.

Scope and duration of procurement eligibility

AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit with product codes ZIK-1111 and ZIK-1112, manufactured by Bioneer Corporation is considered to be eligible for WHO procurement. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

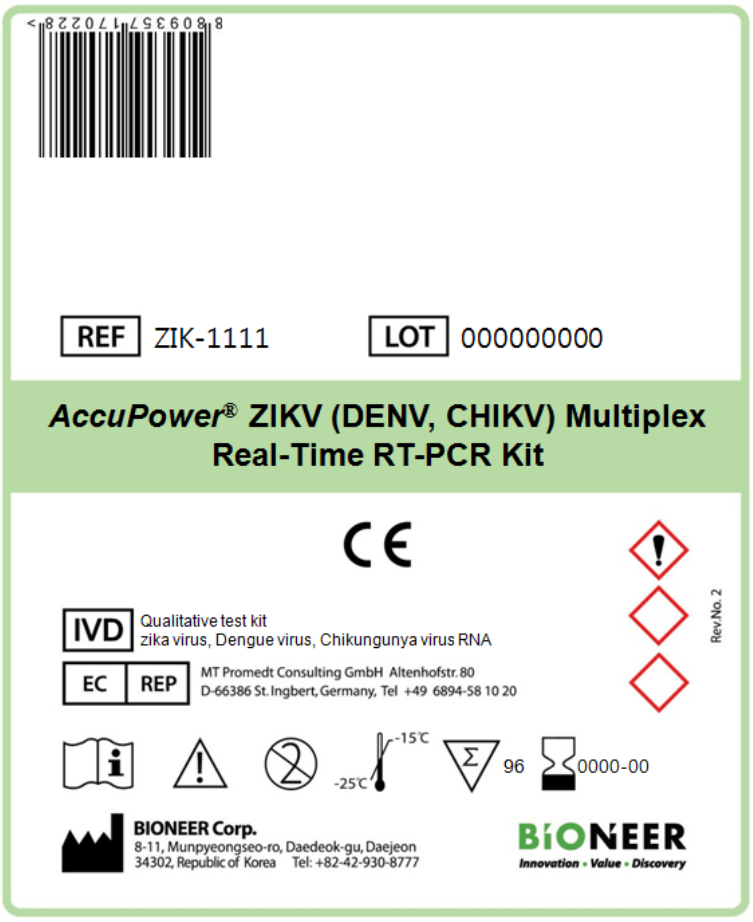
As part of the on-going requirements for listing as eligible for WHO procurement Bioneer Corporation must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Bioneer Corporation is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days of receipt. Furthermore, WHO will continue to monitor the performance of the assay in the field.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality and performance comes to WHO's attention during post-market surveillance activities.

1. Labels

1.1 Box Labels

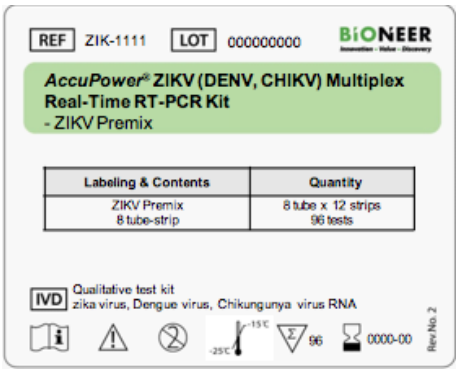
1.1.1 ZIK-1111



1.2 Reagent Labels

1.2.1 ZIK-1111

Premix



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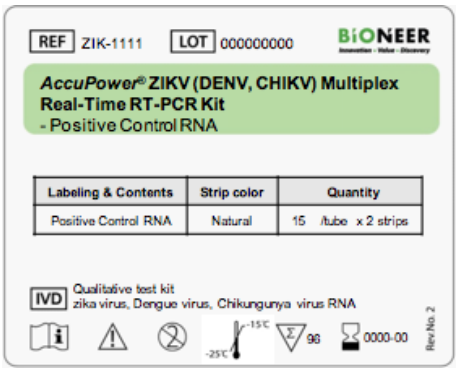
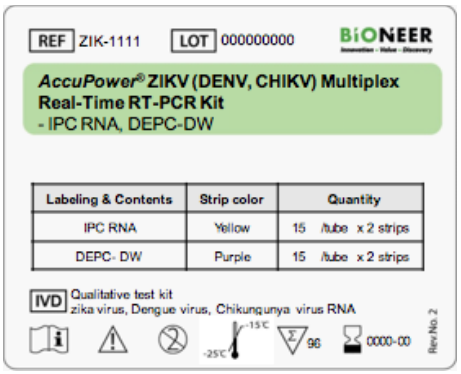


IPC, DEPC-DW (NTC)



DEPC-DW

SL Buffer

2. Instructions for Use

User's Guide Version 1.4 (2016-10)

User's Guide

CE

AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

REF ZIK-1111

IVD Qualitative test kit
Zika virus, Dengue virus, Chikungunya virus RNA

EC REP MT Promedt Consulting GmbH Altenhofstr. 80
D-66386 St. Ingbert. Germany. Tel +49 6894-58 10 20

BIONEER
Innovation • Value • Discovery

AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

User's Guide

Version No.: 1.4 (2016-10)

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

Bioneer Corporation
8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon
34302, Republic of Korea
Tel: +82-42-930-8777
Fax: +82-42-930-8688
Email: sales@bioneer.co.kr
www.bioneer.co.kr

Safety Warnings and Precautions

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, these kits and human sourced specimens are considered potentially infectious and should be handled using safe laboratory procedures and in accordance with OSHA Standard on Bloodborne Pathogens, the Biosafety in Microbiological and Biomedical Laboratories, CLSI Document M29-A3, and other appropriate biosafety practice. Decontamination and disposal of all specimens, reagents and other materials should follow the federal, state and local regulations, as deemed appropriate for the country.

Trademarks

AccuPower® is a registered trademark of BIONEER Corporation, Republic of Korea. *ExiStation*™, *Exicycler*™ 96, *ExiSpin*™ and *ExiPrep*™ are trademarks of BIONEER Corporation, Republic of Korea.

FAM, TAMRA and TET are trademarks of Applera Corporation.

Texas Red is a registered trademark of Molecular Probes Corporation.

Cy5 is a registered trademark of Detection Systems Corporation.

Excel™ is a trademark of Microsoft Corporation.

TABLE OF CONTENTS

1. INTENDED USE

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is an *in vitro* diagnostic kit designed for the qualitative detection of Zika, Dengue and Chikungunya virus RNA in human serum and plasma, and Zika virus RNA in urine, using the *ExiPrep*™ Instrument for automated nucleic acid preparation and the *Exicycler*™96 Instrument for nucleic acid amplification and detection.
The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is intended to aid in the diagnosis and management of patients with potential Zika, Dengue and Chikungunya virus infection.

2. INTRODUCTION

Mosquito-borne diseases are transmitted by mosquitoes. Over one million people die from these diseases every year. There are various causes of mosquito-borne diseases such as bacteria, viruses and parasites. Especially some virus-mediated mosquito-borne diseases tend to be specific to a species of mosquito. Among the variety of viruses which result in mosquito-borne diseases, 3 types of viruses are listed below;
Zika fever is caused by the Zika virus (ZIKV), a member of the *Flaviviridae* virus family. The first detection of ZIKV in human was in 1954 in Nigeria and almost all ZIKV infection had occurred in Africa and Southeast Asia until 2007. In 2007, on the island of Yap in the Federated States of Micronesia, the first outbreak was reported. ZIKV is transmitted via mosquitoes, the *Aedes* genus, primarily. Symptoms of Zika fever are headaches, maculopapular rash, fever, conjunctivitis, and arthralgia. There are no vaccines or drugs for ZIKV currently.
Dengue fever is caused by the Dengue virus (DENV), a member of the family *Flaviviridae*, genus *Flavivirus*. It is serious disease of the Americas, Asia and Africa. *A. aegypti* and *A. albopitus* are the vectors of DENV. Dengue Infection results in acute fever (Dengue fever), headache, muscle pain, joint pain, skin rash and loss of appetite. There currently are no developed drugs for Dengue.
Chikungunya is a disease caused by the chikungunya virus (CHIKV). It has been present in the developing world frequently. Since 2004, it has occurred in Asia, Europe and the Americas. Mosquito, genus *A. albopictus* and *A. aegypti,* is vector of CHIKV. Features of chikungunya are sudden onset of fever and joint pains. Currently, there are no treatments for chikungunya.
There are currently no definitive treatments available for these diseases and early virus detection is critical in managing symptomatic patients. These viruses can be diagnosed by either serological tests or reverse transcription polymerase chain reaction (RT-PCR). Serological testing has limitations as it exhibits cross-reactions with other *flaviviruses*. RT-PCR is highly sensitive and specific in detecting mosquito-borne disease.

3. FEATURES

Real-time PCR involves the selective amplification of a target sequence 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 through the 5' – 3' exonuclease activity of the DNA polymerase during PCR, the fluorescent reporter molecule emits light signal with specific wavelength within the visible spectrum.

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit uses this real-time RT-PCR to generate amplified product from the RNA genome of Zika, Dengue and Chikungunya viruses in clinical specimens. The amount of Zika, Dengue and Chikungunya target sequence that is present at each amplification cycle is proportional to the signal generated from the fluorescent-labeled oligonucleotide probes bound to the amplified target. An RNA sequence that is unrelated to the Zika, Dengue and Chikungunya target sequence serves as an internal positive control (IPC).

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is based on Bioneer's Dual Hot Start technology in detecting RNA target from clinical samples with high sensitivity and specificity. Dual-HotStart™ eliminates non-specific cDNA synthesis and non-specific DNA amplification and allows for the most sensitive one-step RT-PCR assays currently available.

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is also designed to maximize reproducibility and ease-of-use by vacuum-drying all PCR reagents, including primers, probes, DNA polymerase, dNTPs and salts, with Bioneer's proprietary activity-preserving stabilization technology. The primer-probe set is designed to exhibit maximum amplification efficiency using our bioinformatics algorithms. They are matched to our *AccuPower*® Diagnostic Kits to be compatible with *AccuPower*® Diagnostic Kit series.

4. CONTENTS AND RELATED INSTRUMENTS

4.1. Contents of the Kit

AccuPower® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit (Cat. No. ZIK-1111)

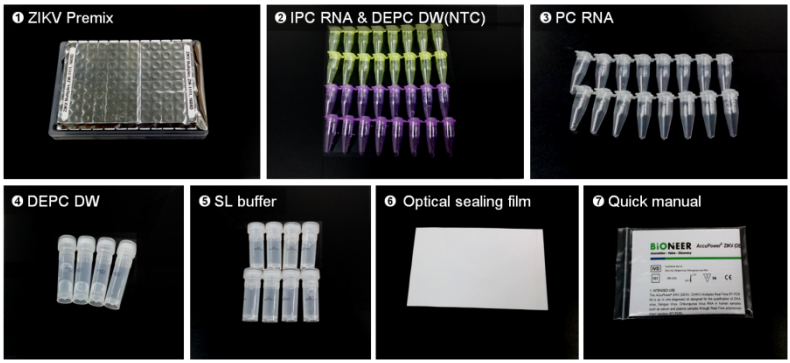


Table 1. Contents of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

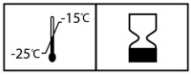
Reagents		Unit	Quantity
①	ZIKV Premix	1 Aluminum Foil Bag	8 tubes x 12 strips (96 test)
②	PC[a] RNA	15 ㎕ / tube (Natural 8-tube strip)	8 tubes x 2 strips (16 tubes)
③	IPC[b] RNA	15 ㎕ / tube (Yellow 8-tube strip)	8 tubes x 2 strips (16 tubes)
	DEPC-DW (NTC[c])	15 ㎕ / tube (Purple 8-tube strip)	8 tubes x 2 strips (16 tubes)
④	DEPC-DW	1800 ㎕ / tube	4 tubes
⑤	SL Buffer	1800 ㎕ / tube	8 tubes
⑥	Optical Sealing Film	-	1 sheet
⑦	Quick Start Guide	-	1 sheet

a : Positive Control (PC), b : Internal Positive Control (IPC), c : No Template Control (NTC)

4.2. Related Instruments

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is optimized for use with BIONEER's *ExiStation*™ Universal Molecular Diagnostic System. For detailed operating instructions, please refer to the *ExiStation*™ *User's Guide*.

5. STORAGE AND EXPIRATION DATE



The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit should be stored between −25°C and −15°C, away from UV/sunlight. Repeated thawing and freezing (more than once) of the components should be avoided, as this may reduce assay performance. If intermittent use of the kit is expected, only those components that will be used should be removed from the freezer.

Once opened, any unused reagents must be discarded. Do not store any of the reagents including PCR premix at temperatures outside of the recommended range. When stored properly, the kit's stability is guaranteed until the expiration date printed on the label.

6. MATERIALS REQUIRED BUT NOT PROVIDED

- Instrumentation and Software
  - *ExiStation*™ Universal Molecular Diagnostic System (Cat. No. A-2200)
    : Integrated platform of Real-Time PCR instrument and nucleic acid extraction Instrument
  - *Exicycler*™ 96 Real-Time Quantitative Thermal Block (Cat. No. A-2060)
  - *ExiPrep*™ 16 Dx (Cat. No. A-5050)
  - *ExiSpin*™ (Cat. No. A-7040)
  - *ExiStation*™ Manager Software
- Disposable gloves, powerless
- Appropriate adjustable volumetric pipette set
- Sterilized pipette tips with filter barriers
- 1.5 ml micro tubes or 15 ml conical tubes
- Other reagents required but not provided
  - *ExiPrep*™ Dx Viral RNA Kit (Cat. No. K-4473)

7. WARNING AND PRECAUTIONS

7.1 General Precautions

- For *In vitro* Diagnostic Use Only

- This Kit is for use only with human serum, plasma and urine specimens.
- Do NOT pipette by mouth.
- DO NOT eat, drink or smoke in laboratory work areas.
- *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit should be used with *Exicycler*™ 96 Real-Time Quantitative thermal block.
- Wear protective disposable gloves, laboratory coats, eye protection and mask when handling specimens and kits.
- Avoid microbial and ribonuclease contamination of reagents when removing aliquots from control tubes.
- The use of sterile disposable pipettes and RNase-free pipette tips is recommended.

- DO NOT reuse opened reagents, nor mix reagents from different production lots.
- DO NOT pool controls form different lots or from different vials of the same lot.
- DO NOT use a kit after its expiration date.
- Avoid microbial contamination of kit components while preparing specimens.
- Please read this *User's Guide* before use.
- DO NOT change the protocol as described in this *User's Guide*.
- Clinical samples and their derivatives should be stored in a separate location/freezer from where the rest of the kit components are stored.
- All kit components should be allowed to slowly thaw for at least 10 minutes, and then vortexed for about 5 seconds and centrifuged another 5 seconds at 2500rpm, before initiating an experiment.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- All positive controls (PC) should be added in a physically separate location from where the premix is reconstituted.
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulation.

7.2 Special Precautions

– Handling Precautions

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is only for use with human plasma and serum (EDTA) specimens and urine specimens that have been handled and stored in capped tubes.

During preparation of sample, compliance with good laboratory practice is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of ribonuclease (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.

Amplification reactions such as PCR are sensitive to accidental introduction of products from previous amplification reactions. Incorrect results can occur if either the clinical specimen or the Real-Time RT-PCR kit used in the amplification step becomes contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR and complying with good laboratory practice.

– Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips are for single use only. Clean and disinfect specimen spillage using the recommended cleaning solution.

– Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNases contamination, cross-contamination between samples and inhibition

- Wear appropriate personal protective equipment at all times.

- Use powder-free gloves.
- Change gloves after having contacts with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all sample and reagent pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.

8. PROTOCOL

8.1 Preparation

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

8.1.1 Work Areas

All sample preparation and *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit testing should be performed in the Basic-Biosafety Level 2 (BSL-2).

Sample handling area must be dedicated to processing samples such as specimens and positive controls, and to adding processed specimens and controls to the sample loading tube. All reagents used in the sample handling area should remain in the area at all times. Supplies including laboratory coats, pipettes, pipette tips and vortexers used in the sample handling work area also must remain in this area and not be moved to the amplification handling work area. Do not bring amplification product into the sample handling work area. Sample handling should be conducted within a negatively pressurized biosafety cabinet where air flows inwards to prevent contamination.

Sterilized containers such as buffer cartridges included with the kit (*ExiPrep*™ Dx nucleic acid extraction kit series) should be opened only in a positively pressurized environment, where air flows outwards, to prevent environmental contaminants from breaking the sterile barrier.

All materials (specimens, reagents and other potentially biohazardous infectious materials) should be handled in a manner that minimizes the chance of potential contamination.

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used. The pipette tips are for single use only. Clean and disinfect specimen spillage using recommended disinfectant. Please refer to Chapter 11, for more information.

Note: Autoclaving the sealed PCR Premix tube will not degrade the amplified product but may contribute to the release of, thus contamination by the amplified product when tubes are opened.

8.2 Specimen

We recommend RNA extracted from serum, plasma and urine samples.

All samples should be treated as potential biohazards.

8.2.1 Specimen Collection

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is optimized for RNA extracted from serum, plasma and urine samples. Blood should be collected in sterile tubes using EDTA (lavender top) as the anticoagulant or in SST® Serum Separation Tubes. All samples should be kept in preservative-free containers.
Separate plasma or serum from whole blood within 24 hours of collection by centrifugation at 800g to 1600g for 20 minutes at room temperature (15°C to 30°C). Transfer plasma or serum to a sterile polypropylene tube, such as 1.5ml or 2ml microtubes.

8.2.2 Specimen Transport

Transportation of whole blood, plasma, serum or urine must comply with country, federal, state and local regulations for the transport of etiologic agents. Specimens should be transported in a shatterproof transport container to prevent potential infection from sample leakage. Specimens should be packaged and labeled in compliance with applicable regulations.

8.2.3 Specimen Storage

Separated serum or plasma and aliquoted urine specimens can be stored between 2°C and 8°C, for up to 5 days. For longer period of storage, samples should be stored between -20°C and -80°C in small aliquots to avoid repeated freeze/thaw cycles.

8.2.4 Interfering Substances

Heparin (≥ 10 IU/㎖) is a known inhibitor of PCR. Samples that have been collected in tubes containing heparin should not be used. In addition, samples from heparin-treated patients should not be used. Clinical samples may contain substances which interfere with PCR. For efficient PCR, such inhibitors must be removed during the RNA extraction and purification process.

8.3 *ExiStation*™ Procedure

The *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit is designed for use with *ExiStation*™ Universal Molecular Diagnostic System.

8.3.1 Work Flow

When using the kit with *ExiStation*™ system, both nucleic acid extraction and PCR should be conducted according to the protocol described in the *ExiStation*™ *User's Guide*. PCR can be performed without additional steps for preparing PCR mixture when using with *ExiStation*™ system. After PCR is completed, the data can be automatically analyzed through *ExiStation*™ Manager software. For detailed operating instructions, please refer to the *ExiStation*™ *User's Guide*.

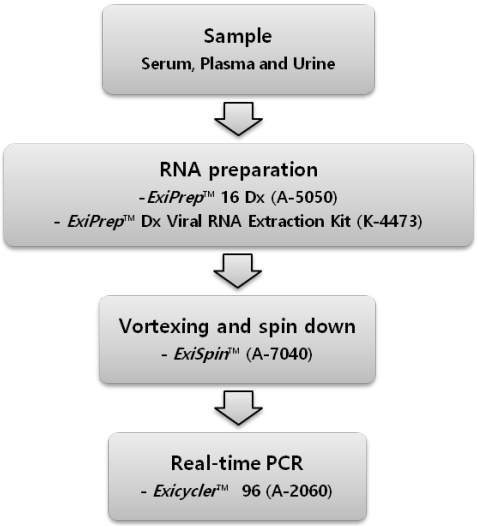


Fig. 1 Work flow

8.3.2 Nucleic Acid Extraction

A. Precautions

The *ExiStation*™ Universal Molecular Diagnostic System utilizes automated nucleic acid extraction on the *ExiPrep*™16 Dx instrument with *ExiPrep*™ Dx Viral RNA Kit. For further information on the extraction, refer to the *ExiPrep*™16 Dx *User's Guide.*

B. Preparation

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

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

Fig. 2 *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. 3 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). 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.

Fig. 4 LCD screen of *ExiPrep*™16 Dx

Fig. 5 Load button of *ExiStation*™ Manager Software

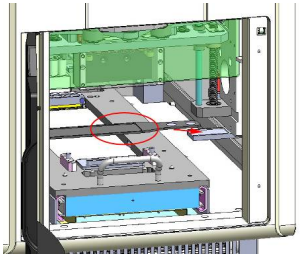


Fig. 6 Mounting the Contamination Shield

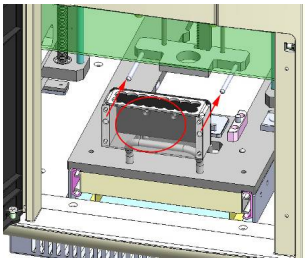


Fig. 7 Mounting the Tip Protector

5) The *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 – after the PCR run, present experiment information and sample result information
Configuration – software set-up information (accessible only by manufacturer)
Version – present software version

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

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

7) Click the pull-down arrow for 'Diagnosis Kit 1'. A popup will appear.

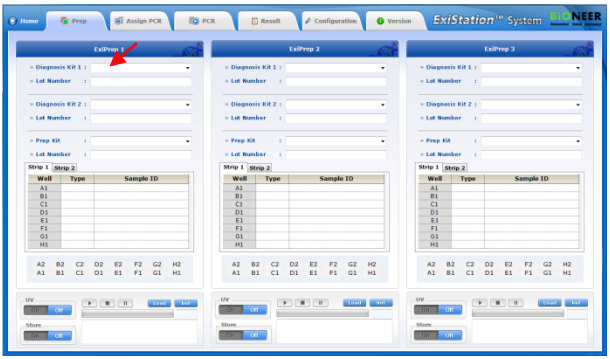


Fig. 9 '**Prep**' Pop-up window of *ExiStation*™ Manager software

8) Select 'ZIK-1111' from the pull-down menus. The appropriate '**Prep Kit**' for the selected diagnostic kit will be automatically assigned.

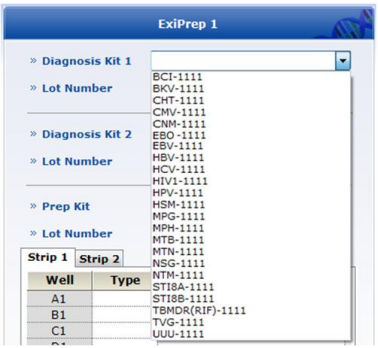


Fig. 10 Selection of Diagnostic kit

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

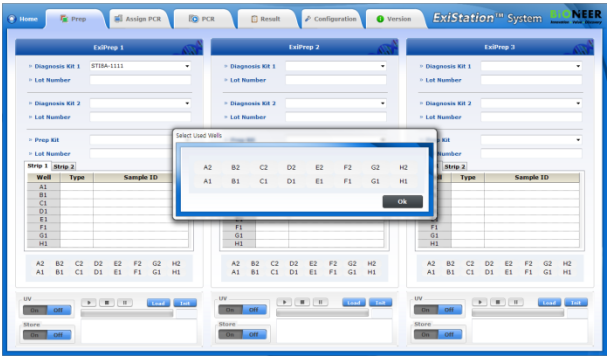


Fig. 11 Exception of the used Buffer Cartridge well

10) The program will automatically allocate the NTC and PC wells. The default setting is one (1) each of NTC and PC.

11) Input each lot number of the diagnostic kit and the prep kit.

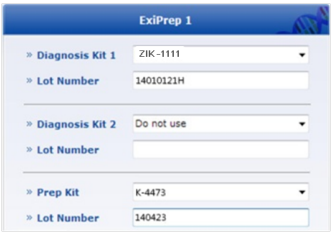


Fig. 12 Entering lot number

12) Click the 'Sample ID' column and enter the sample information by using a barcode reader (optional) or a keyboard.

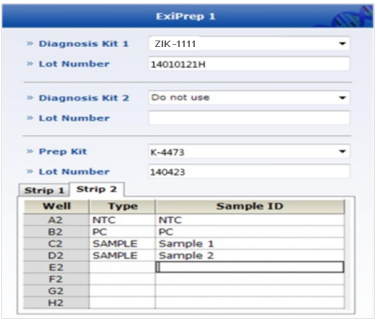


Fig. 13 Enter Sample ID

C. Control and sample loading

The process of loading controls and samples is as follows:

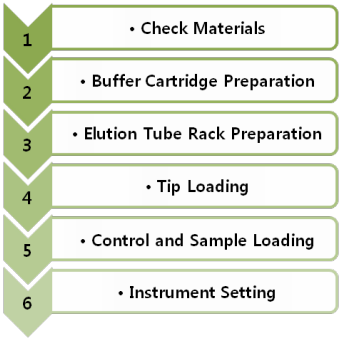


Fig. 14 Work flow of nucleic acid preparation using *ExiPrep*™16 Dx

Buffer Cartridge

1) Check that all necessary materials and accessories are present before proceeding.

Category	Contents
Prep tools	Setup Tray Hole Punch Sample Tube Rack Elution Tube Rack Disposable Tip Rack
Accessories	Buffer Cartridges ① and ② Sample Loading Tubes Disposable Tips Elution Tubes Elution Tube Caps Protection Cover Waste Tray Diagnostic kit tubes Contamination Shield Filter Papers

Table 2. Prep tools and consumables

2) Clean the surface (preferably a positive pressure BSC) where work will be performed.
Note: Clean the surface with 70% EtOH or 5% nitric acid solution before and after use in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.
Note: Turn off the UV lamp while using the BSC.

3) Remove the shrink-wrap enclosing both Buffer Cartridges ① and ② then remove the acryl lids in a positive pressure BSC.
Note: Inspect the wells of the Buffer Cartridges and make sure all liquids are at the bottom of the wells.

4) Punch the film with the Hole Punch according to the layout mapped on the software.
Note: Since improper punching of film may cause malfunction of the instrument, the film should be pushed to fit perfectly inside of each well.

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

Elution Tube Rack (*AccuPower*® Diagnostic Kit Tube)

1) Take the necessary number of strips of the Diagnostic Kit Tubes from the freezer. Remove the foil covering the tubes.

2) 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.

Note: You MUST make sure that the diagnostic tubes are marked so they can be identified during the process.

Note: 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. 15 Inserting the *AccuPower*® Diagnostic Kit tubes into Elution Tube Rack

3) Fasten the Protection Cover onto the Elution Tube Rack.

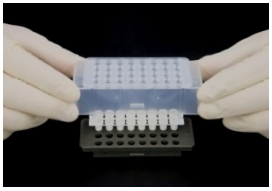


Fig. 16 Protection Cover

4) Load the Buffer Cartridges and Elution Tube Rack into the Setup Tray on the work table.

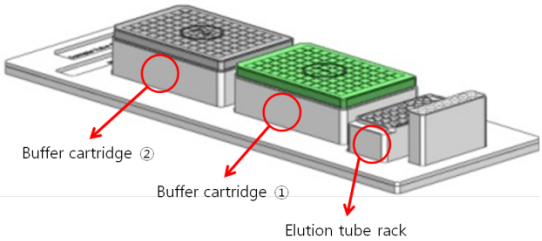


Fig. 17 Loading the Buffer Cartridge and Elution Tube Rack into the Setup Tray on the work table.

Tip Loading

1) Fill the appropriate number of Disposable Tips in the Disposable Tip Rack.

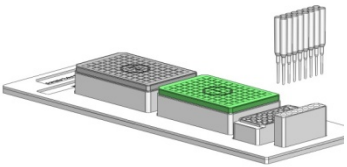


Fig. 18 Inserting the Disposable Tips into the Disposable Tip Rack

Control and Sample Loading

1) Clean the negative pressure Biosafety Cabinet (BSC) on which the nucleic acid extraction preparation will be performed.
   Note: Clean the surface with 70% EtOH or 5% nitric acid solution before and after use in order to prevent contamination. After each use, turn on the UV lamp to eliminate contaminants.
   Note: Turn off the UV lamp while using the BSC.

2) Add 400 ㎕ of Sample Loading (SL) Buffer, 1x PBS or normal saline into pretreated samples and completely dissociate the cell pellet using a pipette.

3) Take out the necessary number of Sample Loading Tubes from the magazine (shown below) and insert them into rack holes.

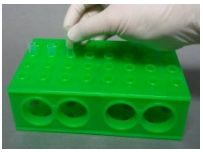


Fig. 19 Inserting the Sample Loading Tube into a rack

4) Take the original clinical sample containers or Sample Loading (SL) buffer (for NTC and PC) and pipette into the Sample Loading Tubes by following steps 5) through 8).

5) For the tube that is assigned as control (NTC and PC), add 400 ㎕ Sample Loading (SL) buffer. (supplied with the *AccuPower*® Diagnostic Kit)

6) Additionally add 5 ㎕ of PC only into the appropriate PC wells. (supplied with the *AccuPower*® Diagnostic Kit)

7) Move the filled Sample Tube into the Sample Tube Rack.
Note: Insert the Sample Tubes vertically to prevent spilling.

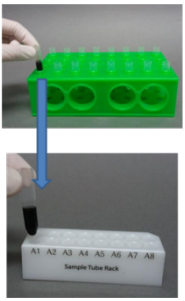


Fig. 20 Moving the Sample Loading Tube into the Sample Tube Rack

8) Uncap the original clinical sample container and pipette 400 ㎕ of sample into the next available Sample Loading Tube. Move the Sample Loading Tube into Sample Tube Rack after pipetting each sample.

9) Repeat step 8) until all samples are loaded.
Note: If for any reason glove or tip contamination by sample is suspected, immediately exchange gloves or a tip to prevent contamination of samples.

10) Take the Sample Tube Rack and load onto the Setup Tray.

11) Place the Waste Tray onto the Setup Tray.

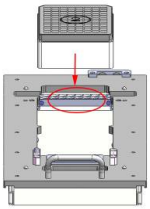
Safety Caution

- When handling the specimen, clean and disinfect all spillage immediately after the occurrence using the recommended solutions listed in 11. **RECOMMENDATION FOR MAINTENANCE OF THE LABORATOTY ENVIRONMENT AND INSTRUMENT**
- At the end of each preparation step, discard all used contents of the kit such as positive control, internal positive control and SL buffer.

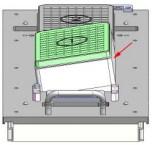
Loading the Instrument

1) Open the door of the *ExiPrep*™16 Dx (A-5050) and pull the Base Plate out completely.

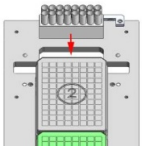
2) Starting from the Buffer Cartridges, place each component one-by-one into the base plate as described below.



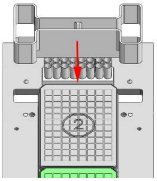
A. Place the Buffer Cartridge ② on the heating block of the base plate.
Note: If Buffer Cartridge ② is not properly placed on the heating block, it may cause an experiment failure or an instrument malfunction.



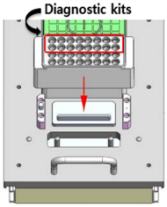
B. Place the Buffer Cartridge ① on the base plate.
Note: Place the Buffer Cartridge ① by putting the cartridge to the left side of the base plate and press right-hand side of the cartridge to press the cartridge to secure.



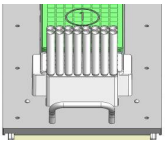
C. Place the Sample Tube Rack on the base plate.
Note: Be careful that the Sample Tube Rack's front and rear sides are properly placed.



D. Place the Waste tray in between the Sample Tube Rack and the Buffer Cartridge ②.
Note: Be careful not to tip over the Sample Tube Rack.



E. Place the Elution Tube Rack on the base plate.
Note: Check the Protection Cover is properly secured on the Elution Tube Rack.



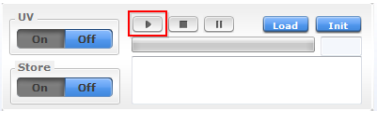
F. Place the Disposable Tip Rack on the base plate.

Note: Make sure the tips, holes and tubes are in alignment.

3) Remove the acryl lids from the Buffer Cartridges.
Note: Make sure the acryl lids of the Buffer Cartridges are removed and all components are in the correct positions.

4) Slide the base plate in and close the door of the *ExiPrep*™16 Dx.
Note: When slide the base plate in, gently push the base plate not to spill the samples and reagents.

5) Click the 'RUN (▶)' button of the *ExiStation*™ Manager Software. Double check whether all accessories for the extraction are loaded properly according to the 'Check ExiPrep Setting' list and check the boxes. Click 'OK' button to initiate the prep process.
Note: Nucleic acid extraction process takes between 80 minutes and 100 minutes depending on the type of nucleic acid.
Note: If any error messages appear during the extraction process on *ExiPrep*™16 Dx LCD, contact your local Bioneer office for technical assistance. In locations where local office is not accessible, please contact Bioneer Corporation directly. Contact information can be found on the last cover page of the User's Guide.



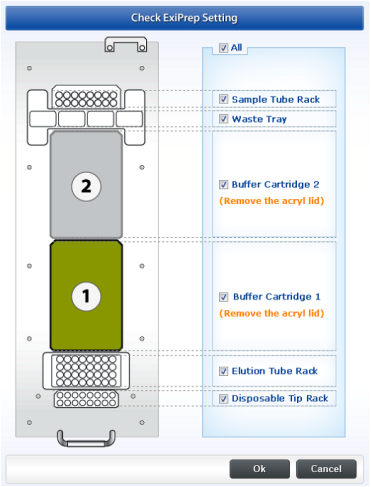


Fig. 21 Click the 'RUN' button on *ExiStation*™ Manager software

8.3.3 PCR Preparation

In order to logically process the samples in a single real-time PCR run without confusing the order or identity of samples, please follow the instructions below.
Note: Initiate PCR preparation process when nucleic acid extraction is finished.

1) Click 'Assign PCR' tab and check the box of each 'Prep Work List' to assign PCR position. Checked 'Prep Work List' is assigned to the PCR position corresponding to the prep instrument 1 to 3 in order.

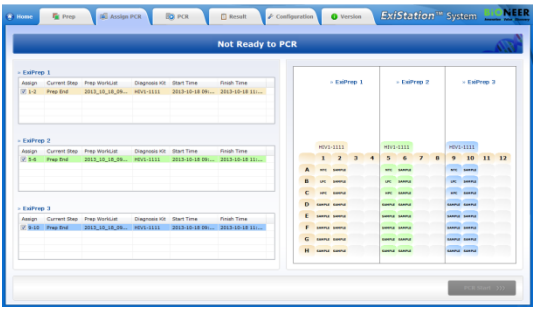


Fig. 22 'Assign PCR' tab

2) After the nucleic acid extraction process is finished, the cooling block is automatically turned off. Select the 'Prep' tab again and click "Store ON" on the control panel to turn the cooling block on for long-term storage.
Note: Prolonged cooling may cause condensation within the diagnostic tubes. This may lead to diluted reagents and erroneous results. Please remember to take out the Elution Tube Rack within 30 minutes after the extraction.

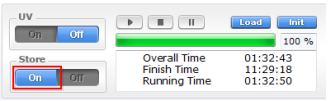
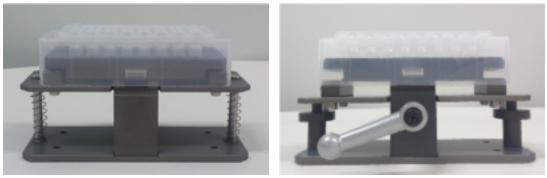


Fig. 23 *ExiPrep*™16 Dx control panel – Store

3) Open the door of the *ExiPrep*™16 Dx (A-5050) after the nucleic acid extraction process is complete, and then remove the Elution Tube Rack.

4) Remove Protection Cover according to Protection Cover Separation Tool utility method.

Note: The Protection Cover Separation Tool had some design improvements. For some users still using the old design tool, included is the old method.



(Picture of the Old (Left) and New (Right) Protection Cover Separation Tool)

Method Using the Previous Model

① Place the Elution Tube Rack from *ExiPrep*™16 Dx on the Protection Cover Separation Tool carefully.

② Place your hands on the Protection Cover and hold the upper side of Separation Tool.

③ Apply gentle pressure on your thumbs and other fingers and gently press down on the Separation Tool.

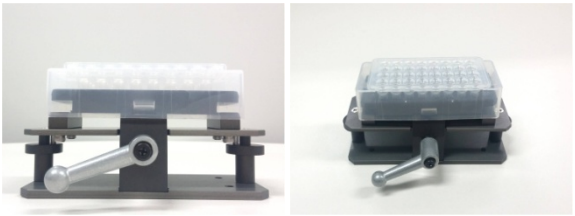
Note: Do not press the Protection Cover.

Note: It helps to wiggle the Separation Tool to facilitate separation of the tubes from the protective film insert within the Protection Cover.

④ Check the Protection Cover and make sure that tubes are not stuck to the Protection Cover.

Method Using the New Model

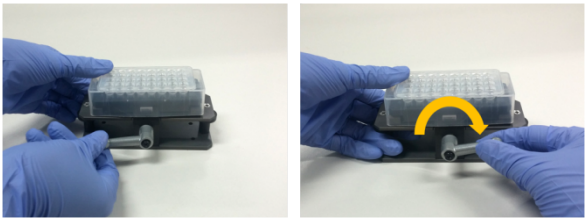
① Take out Elution Tube Rack from ExiPrep™16 Dx and place it on top of the Protection Cover Separation Tool with the lever facing left.



(Picture of the Elution Tube Rack on top of the Protection Cover Separation Tool)

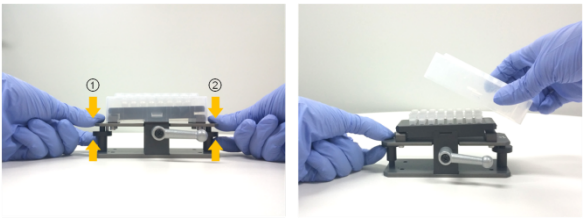
② While firmly holding down the Protection Cover and Separation Tool with one hand, rotate the lever 180 degrees clockwise with the other hand. This will lock the Elution Tube

Rack in place.



(Picture of lever rotation to lock the Elution Tube Rack)

③ Using caution to prevent liquid spillage, press down evenly both sides of the Separation Tool as shown in the picture below. This moves the Protection Cover upwards, separating it from the Elution Tube below.



(Picture of pressing down each side of the Separation Tool and removing the Protection Cover)

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

6) Taking care not to flip the orientation of the tubes, Place the Elution Tube Rack on the PCR Preparation Plate with the corresponding *ExiPrep*™16 Dx number.

Safety Caution

- During the separation of the PCR premix, clean and disinfect all spillage of eluted RNA in the elution tube rack, on the work table or in the work area by using the recommended solutions listed in 11. **RECOMMENDATION FOR MAINTENANCE OF THE LABORATOTY ENVIRONMENT AND INSTRUMENT.**
- Protection cover and disposable tips are for single use only.
- At the end of each preparation step, discard all used consumables such as protection cover, disposable tips, buffer cartridge set (1 and 2), liquid type waste in the waste.

7) Seal the Diagnostic Tubes with the adhesive Optical Sealing Film.
Note: In order to avoid contaminations and invalid results, seal all the tubes thoroughly.

Note: Store the sealed diagnostic tubes at 4°C until use (if the prep reaction is divided into 2 steps, until the end of the 2nd prep reaction).

8) Right before the PCR reaction, completely mix the tube contents using *ExiSpin*™ (A-7040).
Note: *ExiSpin*™ protocol: 2500rpm for 5 sec., Hard vortex for 20 sec. / 20 cycles
Note: Bioneer's PCR premix contains vacuum-dried PCR reagents. Insufficient mixing of the premix could result in invalid PCR results, so mix until the premix is thoroughly dissolved.

9) While the *ExiSpin*™ is operating, you can start the *Exicycler*™ 96.

10) Turn the Standby Power Switch, located at the rear of the instrument ON. The front ring-LED status light should turn on RED.

11) Press the front Operation Power Switch for 3 seconds. A brief self-test sequence will initiate. If the self-test passes, the front ring-LED will blink GREEN with a short beep.

12) Push the Door Switch for 2 seconds to slide the 96-well thermal block out. Insert the reaction tubes in their pre-determined locations. After sample loading is complete, push the Door Switch for 2 seconds to close the door.
Note: Make sure the sample loading configuration is in agreement with the assigned well position.
Note: 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.

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

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.
Note: If un-used wells are present in the Buffer Cartridges, take a lint-free cloth or wipe wet with 70% ethanol and wipe the film surface of the Buffer Cartridges. Replace the acryl lids on the Buffer Cartridges and keep them in a positive pressure BSC until later use.
Note: Cover the used Buffer Cartridges with the acryl lids and discard them according to your local safety regulations.

Caution

Exercise caution when removing the buffer cartridge as the heat block it rests on may still be hot.

15) Press the 'Misc Set' button, remove the Tip Protector and Contamination Shield, and 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.

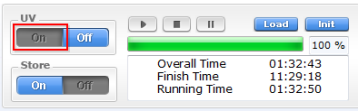


Fig. 24 *ExiPrep*™16 Dx control panel – UV

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

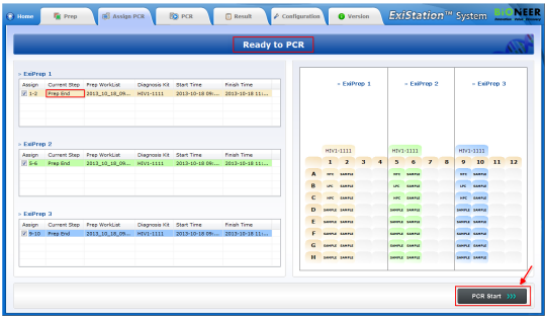


Fig. 25 'Assign PCR' tab – PCR Start

18) A popup will appear prompting the user to enter a Work List Name. Click 'OK' after entering a name to generate a Work List for Real-Time PCR.
Note: Default Work List file path is 'C: > ExiStation_Data > user > GUEST > WorkList'.

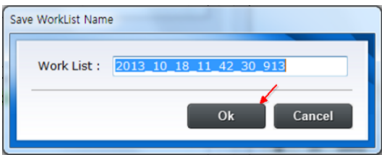


Fig. 26 Save Work List Name

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

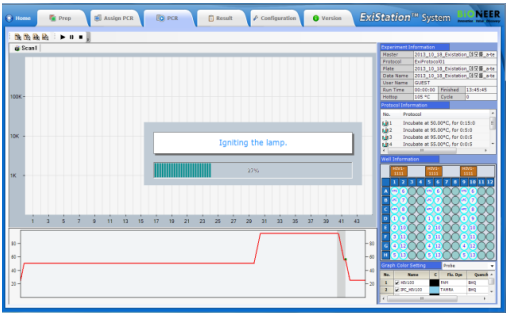


Fig. 27 'PCR' tab

Safety Caution

- The PCR premix tubes are for single use only.
- At the end of each PCR run, discard all used PCR tubes.

8.3.4 Data Analysis

1) After the PCR run is finished, select 'Result' tab to check the results of each samples.
Note: Click 'Analysis' button to open the dedicated analysis popup which presents detailed results including a fluorescence graph.

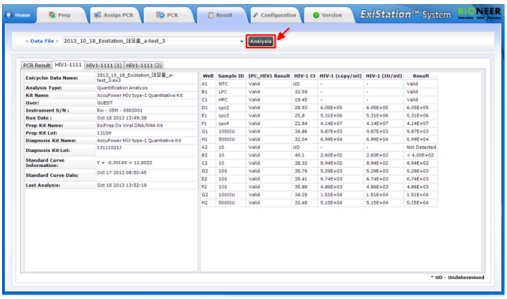


Fig. 28 Result analysis using *ExiStation*™ Manager software

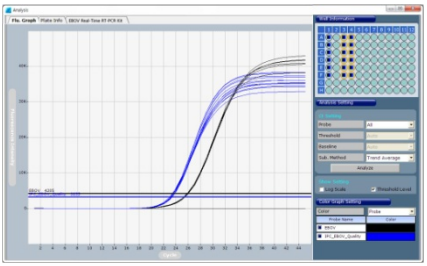


Fig. 29 A popup for result analysis

The result data files are saved in 'C: > ExiStation_Data > user > GUEST > WorkList > relevant data file name' folder.

8.4 Analysis Examples

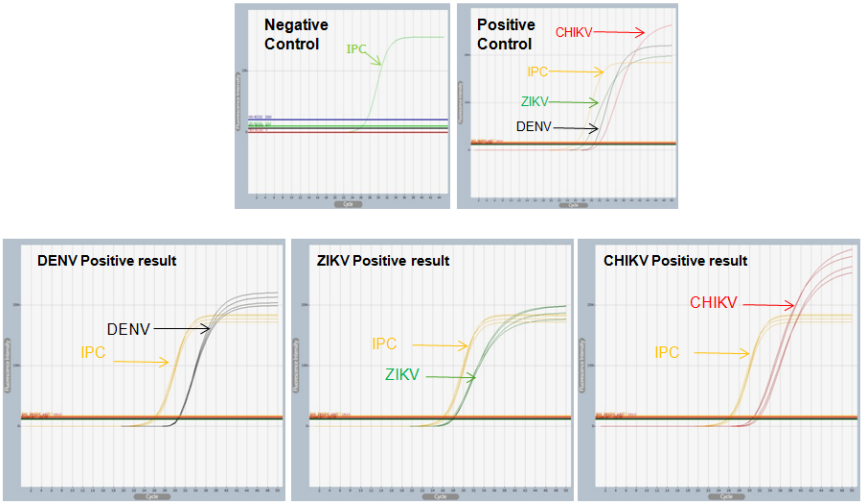


Fig. 30 Result examples

8.5 Interpretation of Results

The test uses 2 wells of each NTC and PC to determine the validity of the experiment, and each reaction includes IPC in wells of samples as well as NTC and PC to check whether PCR works.

NTC: to determine whether the sample is contaminated in the process of sample pretreatment, nucleic acid extraction, and PCR preparation (prevent false-positive error)

PC: to determine whether target RNA is properly amplified (prevent false-negative error)

IPC: to check whether PCR is inhibited by the sample and to determine the amplification of nucleic acids in each well. High concentrations of target DNA can lead to a reduced or absent fluorescence signal of IPC due to PCR competition.

The validity of IPC is determined by Ct value of IPC signal. If its Ct value is within the specified range, it is valid. If the Ct value is out of the specified range, it is invalid. The validity of PC and NTC is determined by Ct value of target signal. If the assay is valid, target Ct will be 'undetermined' in NTC well and PC Ct value will be within its specified range. If the control results are invalid, take measures according to ***User's Guide* 9. Troubleshooting**.

The result of IPC determines the validity of the test, and Ct value of the target signal determines whether the target is 'Detected' or 'Non-detected'.

Cut-off value: to classify results as positive or negative. The cut-off value is determined utilizing statistical technique, probit analysis. The low value of confidence interval (CI) of LoD is converted into Ct value derived from the LoD test. The cut-off Ct value determines the target RNA detection results as positive with 97.5% probability.

Table 3. Cut-off table of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

Type	ZIKV Ct (TET)	DENV Ct (FAM)	CHIKV Ct (Cy5)	IPC Ct (TAMRA)	Result
NTC	-	-	-	22~31	Valid
PC	**22~33**	**22~33**	**22~33**	**22~31**	**Valid**
Sample	**≤40.6**	**≤39.5**	**≤41.4**	**22~31**	**Positive**

For Example

Sample	+	-	-	+	ZIKV
Sample	-	+	-	+	DENV
Sample	-	-	+	+	CHIKV
Sample	+	+	-	+	ZIKV, DENV
Sample	+	-	+	+	ZIKV, CHIKV
Sample	-	+	+	+	DENV, CHIKV
Sample	+	+	+	+	ZIKV, DENV, CHIKV
Sample	-	-	-	+	Non-detected
Sample	+/-	+/-	+/-	-	Repeat

9. 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 EXPIRATION DATE**
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. • (Mode II) 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.

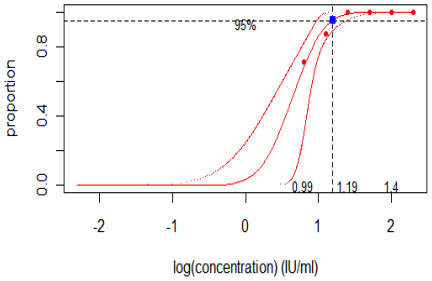
PC invalid results	
If the FAM/TET/ CY5 (PC) fluorescence signal was undetermined.	• The kit may have spoiled, due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE** • 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 EXPIRATION DATE, 7. General Precautions** • PCR Protocol error ☞ Review your reaction preparation procedure. Confirm the amount of PC used in a single well. See ***User's Guide*** **8.4.2 PCR Preparation, 8.5.2 PCR Preparation** • There may have been a pipetting error. ☞ Review the pipetting technique and calibration.
No template Control (NTC) invalid results	
If the FAM/TET/ CY5 (PC) 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 EXPIRATION DATE** • 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*** **8.4.2 PCR Preparation, 8.5.2 PCR Preparation** • There may have been a pipetting error. ☞ Review the pipetting technique and calibration.

10. SPECIFICATION

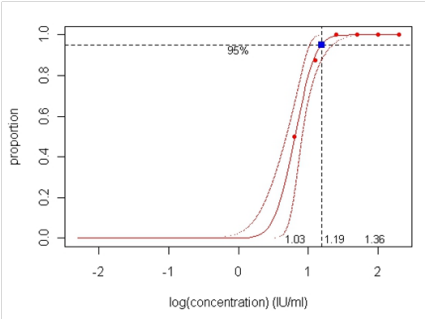
10.1 Analytical Sensitivity (Limit of Detection (LoD))

Viral RNA extracted from Dengue virus type 2 (ATCC® VR-1584™), Chikungunya virus (ATCC® VR-64™) and Zika virus (ATCC® VR-84™) was used to determine LoD of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit. The ZIKV, DENV and, CHIKV RNA concentration of 200 copies/㎖ was serially diluted 2-fold to 6.25 copies/㎖ to prepare 6 different test concentrations of RNA. Twenty-four replicates for each DNA concentration were tested, and the proportion of positive results obtained from each concentration was subjected to probit analysis. The probit analysis determined that the concentration of ZIKV, DENV and, CHIKV RNA detected with 95% probability was 15.48, 15.8, 18.6 copies/㎖.

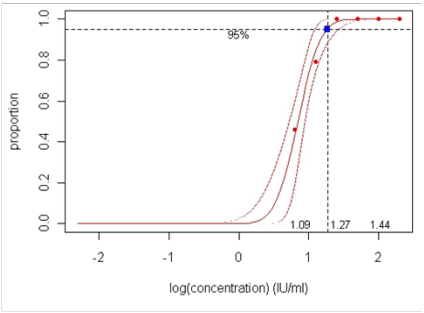
Matrix: Chemicon Human Serum, Normal (Cat. No. S1-LITER)



[Zika Virus]



[Dengue Virus]



[Chikungunya Virus]

Fig. 34 Probit analysis of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

LoD of each matrix Results.

Matrix / Target	Serum	Plasma	Urine(only for ZIKV)
ZKIV(cp/㎖)	15.48 cp/㎖ (95% CI 9.77~25.11 cp/㎖)	15.85 cp/㎖ (95% CI 10.23~25.54 cp/㎖)	21.88 cp/㎖ (95% CI 14.12~36.67 cp/㎖)
DENV(cp/㎖)	15.8 cp/㎖ (95% CI 10.7~22.9 cp/㎖)	19.05 cp/㎖ (95% CI 12.3~28.84 cp/㎖)	N/A
CHIKV(cp/㎖)	18.6 cp/㎖ (95% CI 12.3 ~ 27.55 cp/㎖)	19.5 cp/㎖ (95% CI 13.49~28.18 cp/㎖)	N/A

10.2 Dynamic Range & Linearity

Positive RNA was used to determine measurement range and linearity. The PC RNA concentration of 6.25 $X10^6$ copies/ml was serially diluted 10-fold to 6.25$X10^1$ copies/ml to prepare 6 different test concentrations of RNA. Three replicates for each RNA concentration were tested. The measurement range (linearity with R^2 values over 0.950) of the ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR assay was between 6.25$X10^6$ and 6.25$X10^1$ copies/ml.

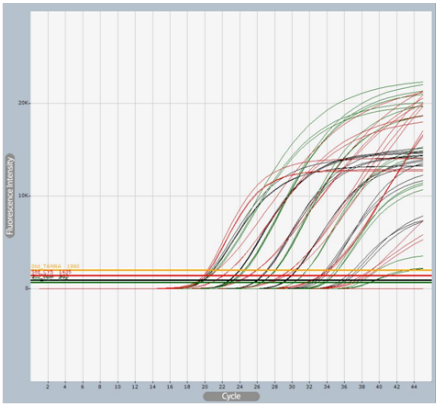


Fig. 35 Measurement range and linearity of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

10.3 Cross Reactivity

A total of 36 species of bacteria, virus, human total RNA were tested for potential cross reactivity in the use of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit. Three replicates of each RNA were run for the evaluation.

Table 4. List of pathogens for cross reactivity test

A. Results for non-related species

No.	Pathogens			AccuPower® ZIKV (DENV, CHIKV) Multiplex RealNegativeTime RTNegativePCR Kit			
		ATCC No. or National code	Conc.(ng/test)	ZIKV signal	DENV signal	CHIKV signal	IPC Signal
1	Bacillus cereus	21366	5	Negative			Positive
2	Enterococcus faecalis	29212	5	Negative			Positive
3	Enterococcus feacium	8043	5	Negative			Positive

4	Listeria monocytogenes	15313	5	Negative	Positive
5	Staphylococcus aureus	25923	5	Negative	Positive
6	Citrobacter freundii	6750	5	Negative	Positive
7	Enterobacter aerogenes	13048	5	Negative	Positive
8	Enterobacter cloacae	11439	5	Negative	Positive
9	Escherichia coli	25922	5	Negative	Positive
10	Escherichia coli	35218	5	Negative	Positive
11	Klebsiella oxytoca	8724	5	Negative	Positive
12	Klebsiella pneumoniae	13883	5	Negative	Positive
13	Morganella morganii	25830	5	Negative	Positive
14	Proteus mirabilis	25933	5	Negative	Positive
15	Proteus vulgaris	13315	5	Negative	Positive
16	Mycobacterium smegmatis	19420	5	Negative	Positive
17	Mycobacterium gordonae	14470	5	Negative	Positive
18	Mycobacterium aubagnense	50186	5	Negative	Positive
19	Mycobacterium szulgai	35799	5	Negative	Positive
20	Mycobacterium terrae	15755	5	Negative	Positive
21	Mycobacterium intracellulare	13950	5	Negative	Positive
22	Mycobacterium massiliense	48898	5	Negative	Positive
23	Mycobacterium chelonae	35752	5	Negative	Positive
24	Ureaplasma parvum	27513	5	Negative	Positive
25	HCV(RNA)	Acrometrix	8,500 IU/ml	Negative	Positive
26	HBV	NIBSC	8,500 IU/ml	Negative	Positive

27	BKV	Acrometrix	8,500 IU/ml	Negative	Positive
28	Human total RNA	Hela cell	100	Negative	Positive
	Human Genomic DNA	Hela Cell	100	Negative	Positive
29	Yellow fever vaccine strain	STAMARIL L5026	100 ul	Negative	Positive
30	West nile virus	AcroMetrix™ WNV Training Panel Cat.No.:950250	10,000 cp/ml	Negative	Positive
31	Parvovirus B19	NIBSC code: 12/208	10,000 cp/ml	Negative	Positive
32	Plasmodium falciparum	NIBSC Code: 04/176	10,000 cp/ml	Negative	Positive
33	HIV-1	NIBSC Code: 10/152	8,500 IU/ml	Negative	Positive
34	HIV-2	NIBSC Code: 08/150	400 IU/ml	Negative	Positive
35	Cytomegalovirus (CMV),	Acrometrix	8,500 IU/ml	Negative	Positive
36	Epstein Barr Virus (EBV),	Acrometrix	8,500 IU/ml	Negative	Positive

B. Results for related species

No.	Pathogens			*AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit			
		ATCC No. or National code	Conc.(cp/ml)	ZIKV signal	DENV signal	CHIKV signal	IPC Signal
1	DENV-1	From KNIH	1,000 cp/ml	Negative	Positive	Negative	Positive
2	DENV-2	VR-1584™	1,000 cp/ml	Negative	Positive	Negative	Positive
3	DENV-3	KBPV-VR-30	1,000 cp/ml	Negative	Positive	Negative	Positive
4	DENV-4	KBPV-VR-31	1,000 cp/ml	Negative	Positive	Negative	Positive
5	ZIKV	VR-84™	1,000 cp/ml	Positive	Negative	Negative	Positive
6	CHIKV	VR-64™	1,000 cp/ml	Negative	Negative	Positive	Positive

10.4 Interfering Substances Testing

Interference testing was performed to determine whether potential interfering substances would affect the ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR assay results. Clinical samples known to be positive or negative for ZIKV, DENV and CHIKV were spiked with high levels of potentially interfering substances and tested. Three replicates were tested for each substance. No interference was observed for the substances listed in Table 5.

Table 5. Interfering substances testing of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

[Zika Virus]

Materials	Conc.	Aver. Ct value			SD	CV(%)	▵Ct
		Only Positive sample	Positive sample +Materials	Negative sample +Materials			
K_2 EDTA	540 mg/dL	31.28	31.04	UD	0.19	1.09	0.24
Citrate	327 M/ml	31.53	31.40	UD	0.23	1.22	0.13
Heparin	3 KU/dL	31.71	31.65	UD	0.24	1.23	0.06
Hemoglobin	200 mg/dL	31.59	31.85	UD	0.23	1.25	-0.26
Cholesterol	500 mg/dL	31.44	31.36	UD	0.23	1.25	0.08
Albumin	14.7 g/dL	31.52	31.47	UD	0.26	1.26	0.05
Bilirubin	25 mg/dL	31.38	31.30	UD	0.29	1.27	0.08

*** UD: Undetermined**

[Dengue Virus]

Materials	Conc.	Aver. Ct value			SD	CV(%)	▵Ct
		Only Positive sample	Positive sample +Materials	Negative sample +Materials			
K_2 EDTA	540 mg/dL	31.13	30.83	UD	0.22	1.20	0.30
Citrate	327 M/ml	30.95	30.82	UD	0.21	1.20	0.13
Heparin	3 KU/dL	31.24	30.84	UD	0.28	1.31	0.40
Hemoglobin	200 mg/dL	30.81	31.07	UD	0.29	1.34	-0.26
Cholesterol	500 mg/dL	30.98	31.13	UD	0.24	1.23	-0.15
Albumin	14.7 g/dL	30.87	30.94	UD	0.26	1.25	-0.07
Bilirubin	25 mg/dL	30.83	31.06	UD	0.32	1.90	-0.23

*** UD: Undetermined**

[Chikungunya Virus]

Materials	Conc.	Aver. Ct value			SD	CV(%)	▵Ct
		Only Positive sample	Positive sample +Materials	Negative sample +Materials			
K_2 EDTA	540 mg/dL	31.65	31.39	UD	0.21	1.12	0.26
Citrate	327 M/ml	31.56	32.02	UD	0.22	1.22	-0.46
Heparin	3 KU/dL	31.70	31.35	UD	0.25	1.25	0.35
Hemoglobin	200 mg/dL	31.61	31.59	UD	0.25	1.25	0.02
Cholesterol	500 mg/dL	31.56	31.26	UD	0.24	1.24	0.30
Albumin	14.7 g/dL	31.62	31.60	UD	0.26	1.26	0.02
Bilirubin	25 mg/dL	31.61	31.44	UD	0.28	1.28	0.17

*** UD: Undetermined**

10.5 Precision

10.5.1 Whole System Failure Rate

96 replicates of low positive sample (2 x LoD concentration) were tested to determine whole system failure rate. All tests were 100% positive, indicating that the multiplex assay of ZIKV, DENV and, CHIKV is robustly stable.

10.5.2 Repeatability

To evaluate the repeatability of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit, within-run, between-run, between-day, and total precision were determined. Two replicates of three different concentrations of Positive Controls (high(5×10^4 cp/㎖), middle (5×10^3 cp/㎖), low (5×10^1 cp/㎖)) per run were tested twice per day for 20days. The standard deviation (SD) of the assay results are shown in Table 6.

Table 6. Precision data of *AccuPower*® ® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

A. Zika

Sample type		Variance Components				
Conc.	No. of Valid Tests	Within-Run SD	Between-Run SD	Between-Day SD	Total SD	CV(%)
High	80	0.175	0.09	0.21	0.32	0.89
Middle	80	0.188	0.07	0.22	0.38	1.22
Low	80	0.213	0.17	0.32	0.49	2.43
Negative		100.0% (80/80) " Not Detected"				

B. DENV

Sample type		Variance Components				
Conc.	No. of Valid Tests	Within-Run SD	Between-Run SD	Between-Day SD	Total SD	CV(%)
High	80	0.135	0.12	0.43	0.42	0.78
Middle	80	0.144	0.09	0.36	0.33	1.19
Low	80	0.181	0.21	0.51	0.52	2.02
Negative		100.0% (80/80) " Not Detected"				

C. CHIKV

Sample type		Variance Components				
Conc.	No. of Valid Tests	Within-Run SD	Between-Run SD	Between-Day SD	Total SD	CV(%)
High	80	0.144	0.18	0.26	0.28	0.98
Middle	80	0.121	0.11	0.21	0.29	1.12
Low	80	0.312	0.43	0.62	0.73	2.24
Negative		100.0% (80/80) " Not Detected"				

10.5.3 Reproducibility

To evaluate the reproducibility of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit, inter-operator, inter-instrument and inter-batch precision were determined. Two replicates of three different concentrations of Positive Controls (high(5×10^4 cp/㎖), middle (5×10^3 cp/㎖), low (5×10^1 cp/㎖)) per run were tested 2 runs per day for 20 days. The SD and coefficient of variation (CV) of the assay results are shown in Table 7.

Table 7. Reproducibility data of *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit

A. ZIKV

Assay	Category	Conc.		
		High	Middle	Low
Inter-operator	Aver.	27.24	30.12	38.73
	SD	0.32	0.38	1.07
	CV(%)	1.09	1.15	2.48
Inter-instrument	Aver.	27.51	30.49	38.91
	SD	0.45	0.66	1.81
	CV(%)	1.21	1.19	3.38
Inter-batch	Aver.	27.19	30.23	38.67
	SD	0.41	0.76	1.12
	CV(%)	1.19	1.13	1.98

B. DENV

Assay	Category	Conc.		
		High	Middle	Low
Inter-operator	Aver.	28.11	31.54	37.95
	SD	0.48	0.57	0.78
	CV(%)	1.65	1.69	1.82
Inter-instrument	Aver.	28.41	31.96	38.49
	SD	0.47	0.62	1.87
	CV(%)	1.63	1.95	3.46
Inter-batch	Aver.	28.21	32.04	38.12
	SD	0.49	0.92	1.09
	CV(%)	1.75	1.96	2.04

C. CHIKV

Assay	Category	Conc.		
		High	Middle	Low
Inter-operator	Aver.	28.45	30.89	39.51
	SD	0.41	0.45	0.93
	CV(%)	1.42	1.38	2.16
Inter-instrument	Aver.	28.12	29.79	38.46
	SD	0.63	0.91	1.69
	CV(%)	1.81	2.03	3.34
Inter-batch	Aver.	28.14	31.06	39.45
	SD	0.51	0.49	0.79
	CV(%)	1.59	1.55	2.21

10.6 Stability

10.6.1 Storage Stability Study

Stability test was performed to evaluate the stability of the *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit. From the results, the validity period of the *AccuPower*® ZIKV (DENV, CHIKV) Multiplex Real-Time RT-PCR Kit was determined as 12 months at -20°C (-25°C to -15°C).

11. RECOMMENDATION FOR THE MAINTENANCE OF LABORATORY ENVIRONMENT AND INSTRUMENT

11.1 Monitoring the Laboratory for the Presence of Contamination

It is recommended that this test be done at least once a month to monitor laboratory environment, including all working surfaces and equipment, for contamination by amplification products. It is very important to test all areas that may have been exposed to processed specimens, controls and/or amplification products. This includes routinely handled objects such as pipettes, *ExiPrep*™ instrument, *Exicycler* ™ 96 instruments, laboratory bench surfaces, microcentrifuges and centrifuge rotors.

1) Add 0.5 mL of RNase-free water to a 1.7 mL RNase-free microcentrifuge tube.
2) Saturate the cotton tip of an applicator (Sterilzed and RNase-free type) with the RNase-free water from the microcentrifuge tube, and wipe clean the area being monitored in a sweeping motion.
3) Place the applicator back into the microcentrifuge tube and swirl it 10 times.
4) Press the applicator tip along the side of the tube to squeeze out the remaining liquid. Discard the applicator.
5) Pipette 0.5 mL of the liquid collected in the microcentrifuge tube to a clean tube and cap the microcentrifuge tube.
6) Test the liquid in the clean tube using the same test procedure for clinical specimen.
7) The presence of contamination is indicated by the detection of Zika, Dengue, Chikungunya nucleic acids in this swab sample.
8) In case contamination is detected, follow the decontamination procedures outlined in 11.2.3. Cleaning Instruction in Case of Contamination.
9) Repeat the process until the laboratory environment, including working surface and equipment, is free of contamination.

11.2 Cleaning the Laboratory

Note: Clean the laboratory, including the working area and instruments, before and after each experiment.

11.2.1 Cleaning the Specimen Handling Area (Biosafety Cabinet and/or Clean Bench)

1) Apply 70% Ethanol on the worktable surface of the biosafety cabinet or clean bench using a squeeze bottle.
2) Wipe clean the area using a clean paper towel.

3) Repeat.

4) Put on U.V. protective glasses or goggles. Turn on the U.V. lamp and irradiate the specimen handling area for at least 30 minutes.

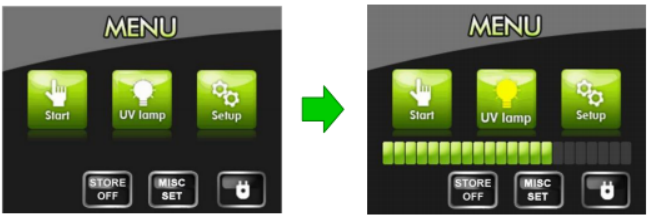
11.2.2 Cleaning *ExiPrep*™ 16 Dx *Before* the Experiment

1) Press the "Main Power" button (①) on *ExiPrep*™ 16 Dx.
2) Press the "STARTING" button on the Main LCD screen (②).

Fig 36. Location of the "Main Power" button and "STARTING" button. on the *ExiPrep*™ 16 Dx instrument

3) Make sure the door is closed.

4) Press the "UV lamp" button on the Main LCD screen. The UV lamp turns on for 15 minutes to sterilize the inside of *ExiPrep*™ 16 DX. The UV lamp turns off automatically.



MENU Screen UV Sterilization Screen

Fig 37. Main LCD screen showing the UV lamp irradiation in progress.

5) *ExiPrep*™16 Dx is ready.

11.2.3 Cleaning *ExiPrep*™ 16 Dx *After* the Experiment

1) After the experiment is finished and *ExiPrep*™16 Dx comes to a complete stop, open the door and slide out the Base Plate.

Fig 38. Sliding out the base plate

2) Taking caution not to spill any liquid waste, lift and remove the Waste Tray from the Base Plate. Discard the liquid waste in the designated biohazard waste container.

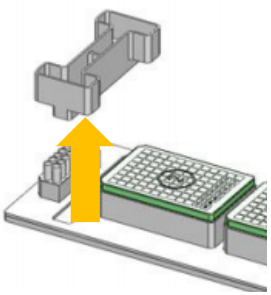


Fig 39. Removing the waste tray

3) Discard the emptied Waste Tray into the designated biohazard waste container.

4) Put the lid back on the Buffer Cartridge 1 and Buffer Cartridge 2. Discard them into the designated biohazard waste container.

   Optionally, If there are unused wells in the Buffer Cartridge, it can be saved for later usage by putting the lid back on and storing it at room temperature.

5) Discard all used Disposable Tips and Sample Loading Tubes into the designated biohazard waste container.

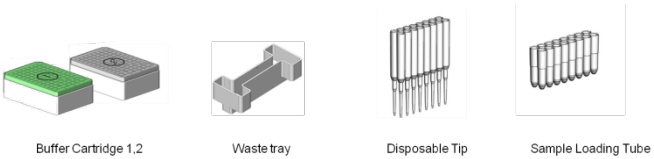


Fig 40. Disposables.

6) Detach the Contamination Shield and Tip Protector from inside. Remove the Sample Tube Rack, Elution Tube Rack and Disposable Tip Rack as well.

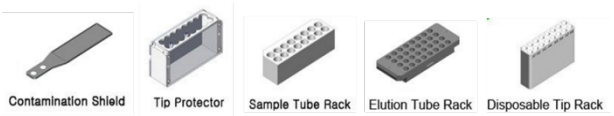


Fig 41. Accessories of *ExiPrep*™ 16 Dx.

Note: To detach the Contamination Shield and Tip Protector, press the "MISC SET" button from the Main Menu. This will move forward the Syringe Block for easier access to its attachment point. Open the door and remove the accessories inside the instrument. Press again the "MISC SET" to move back the Syringe Block to its initial state. Close the door.

Fig 42. "MISC Set" button.

7) Apply 70% Ethanol on the removed accessories using a squeeze bottle and clean each accessory carefully and thoroughly using a clean paper towel.

Note: Before cleaning the Contamination Shield, remove the Contamination Shield Filter Paper.

Note: Tip Protector on the Syringe Block needs careful cleaning.

8) Re-install all cleaned accessories.

Note: To re-install the Contamination Shield and Tip Protector, press the "MISC SET" button from the Main Menu. This will move forward the Syringe Block for easier access to its attachment point. Open the door and re-install the Contamination Shield and Tip Protector. Press again the "MISC SET" to move back the Syringe Block to its initial state.

9) Close the door.

10) Press the "UV lamp" button on the Main LCD screen. UV lamp turns on for 15 minutes to sterilize the inside of *ExiPrep*™ 16 DX. UV lamp turns off automatically

11.2.4 Cleaning *ExiPrep*™ 16 Dx in Case of Contamination

1) If contamination occurs inside of the *ExiPrep*™ 16 Dx instrument, clean the Base Plate using commercially available decontamination reagents such as DNA*Zap*™ or RNA*Zap*™ solutions.

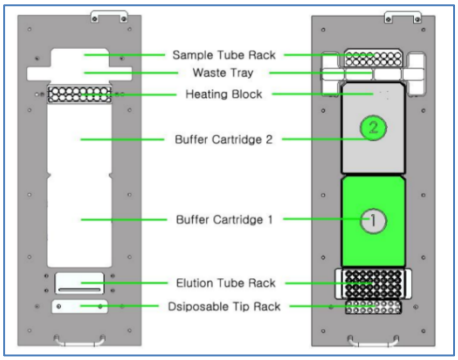


Fig 43. Position of the Base plate in the *ExiPrep*™ 16 Dx instrument.

2) Apply decontamination reagent liberally to the Base Plate and inside wall of the *ExiPrep*™ 16 Dx. Wipe clean using a clean paper towel.

3) Rinse with distilled water and wipe dry with a clean paper towel. Discard all used paper towel immediately.

4) Clean the external surface of the *ExiPrep*™ 16 Dx using commercially available decontamination reagents such as DNA*Zap*™ or RNA*Zap*™ solutions.

5) Detach the Contamination Shield and Tip Protector from inside. Remove the Sample Tube Rack, Elution Tube Rack and Disposable Tip Rack as well.

Note: To detach the Contamination Shield and Tip Protector, press the "MISC SET" button from the Main Menu. This will move forward the Syringe Block for easier access to its attachment point. Open the door and remove the accessories inside the instrument. Press again the "MISC SET" to move back the Syringe Block to its initial state. Close the door.

Fig 42. "MISC Set" button.

6) Apply decontamination reagent liberally on the removed accessories and wipe clean each accessory carefully and thoroughly using a clean paper towel.

7) Rinse with distilled water and wipe dry using a clean paper towel.

Note: Before cleaning the Contamination Shield, remove the Contamination Shield Filter Paper.
Note: Tip Protector on the Syringe Block needs careful cleaning.

8) Re-install all cleaned accessories.

Note: To re-install the Contamination Shield and Tip Protector, press the "MISC SET" button from the Main Menu. This will move forward the Syringe Block for easier access to its attachment point. Open the door and re-install the Contamination Shield and Tip Protector. Press again the "MISC SET" to move back the Syringe Block to its initial state.

9) Close the door.

10) Press the "UV lamp" button on the Main LCD screen. UV lamp turns on for 15 minutes to sterilize the inside of *ExiPrep*™ 16 DX. UV lamp turns off automatically

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13. SYMBOLS

REF	Catalogue number		Temperature limits
IVD	*In vitro* diagnostic medical device		Contains sufficient for test
	Manufacturer		Caution
LOT	Batch code		Expiration date
	Do not reuse		Consult instructions for use
CE	Conformite Europeenne Mark	EC REP	Authorized representative in the European Community

Bioneer Worldwide

Bioneer Corporation

Address	8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
Tel	+82-42-930-8777 (Korea: 1588-9788)
Fax	+82-42-930-8688
E-mail	sales@bioneer.com
Web	www.bioneer.com

Bioneer Inc.

Address	1301 Marina Village PKWY, Suite 110, Alameda, CA 94501, USA
Tel	+1-877-264-4300 (Toll-free)
Fax	+1-510-865-0350
E-mail	orderusa@bioneer.com
Web	us.bioneer.com

Bioneer R&D Center

Address	Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si Gyeonggi-do, 13488, Republic of Korea
Tel	+82-31-628-0500
Fax	+82-31-628-0555
E-mail	sales@bioneer.co.kr
Web	www.bioneer.co.kr

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