For Professional Use Only

Allplex™

Vaginitis Screening Assay

(Cat. No. SD9750X, SD10321Z)

A multiplex real-time PCR assay for the detection of *Lactobacillus* spp., *Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus* spp., *Candida albicans*, *Candida* others and *Trichomonas vaginalis* from genital swab and liquid based cytology specimens.

For use with

1. **CFX96™ Real-time PCR Detection System (CFX96 Manager™ Software-IVD v1.6)**
2. **CFX96™ Dx System (CFX96 Manager™ Dx Software v3.1)**

CE

REF **SD9750X** Σ 100 REF **SD10321Z** Σ 25

IVD **For *in vitro* diagnostic use only**

Seegene Inc.
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EC REP **Medical Technology Promedt Consulting GmbH**
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Not available in the U.S.

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NOTICES

- For *in vitro* diagnostic use only.
- Reliability of the results depends on adequate specimen collection, storage, transport and processing procedure.
- If this product is used with **Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet,** it provides maximum 5 separate runs.
- **AIOS** combines Seegene STARlet sold by Seegene with real-time PCR equipment (CFX96 Dx, Manufacturer: Bio-Rad) and plate sealer (Manufacturer: SAMICK THK) to form an automated linkage structure of nucleic acid extraction to PCR.
- **This test has been validated for the following specimen types: Genital swab and Liquid based cytology.** This test has not been validated for any other types of specimens.
- **Store DNA samples at ≤ -20℃ until use and keep samples on ice during use.**
- Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer period of time.
- Workflow in the laboratory should proceed in a unidirectional manner.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. Use of sterilized aerosol resistant disposable pipette tips is recommended.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use the product after its expiry date.
- Do not reuse all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.
- Please be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive control. To prevent contamination of reagents, use of filter-tips is recommended.

- Use separated and segregated working areas for each experiment.
- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.
- Store positive materials separated from the kit's reagents.
- Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components (product residuals, packaging) can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Expiry date is 12 months from the date of manufacture at ≤ -20℃. Please refer to label for final expiry date.
- Seegene NIMBUS and Seegene STARlet are the same equipment as the Microlab NIMBUS IVD and Microlab STARlet IVD, although the manufacturer is different. Since there are no hardware changes on the device, the test results are the same.
- The brand name of "CFX96™ Real-time PCR Detection System-IVD" is changed to "CFX96™ Dx System". Since there are no hardware changes to the systems, it is expected to obtain the same results from both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software-IVD v1.6". The upgraded software includes enhancements to the "Run" menu. These enhancements do not impact the results of data analysis; therefore, results will be the same.

INTENDED USE

Allplex™ Vaginitis Screening Assay is a qualitative and quantitative *in vitro* test for the single or multiple pathogen detection of *Lactobacillus* spp. (Lacto; *Lactobacillus crispatus, Lactobacillus gasseri* and *Lactobacillus jensenii*), *Gardnerella vaginalis* (GV), *Atopobium vaginae* (AV), *Mobiluncus* spp.(Mob; *Mobiluncus mulieris* and *Mobiluncus curtisii*), *Candida albicans* (CA), *Candida* others (CO; *Candida krusei, Candida glabrata, Candida dubliniensis, Candida parapsilosis, Candida tropicalis* and *Candida lusitaniae*) and *Trichomonas vaginalis* (TV).

- Quantitative detection of *Lactobacillus* spp. (Lacto), *Gardnerella vaginalis* (GV) and *Atopobium vaginae* (AV)
- Qualitative detection of *Mobiluncus* spp. (Mob), *Candida albicans* (CA), *Candida* others (CO) and *Trichomonas vaginalis* (TV)

Allplex™ Vaginitis Screening Assay is intended to aid the diagnosis of vaginal infection in women with a clinical symptom consistent with bacterial vaginosis. Allplex™ Vaginitis Screening Assay should be interpreted in conjunction with other laboratory clinical data from the physicians.

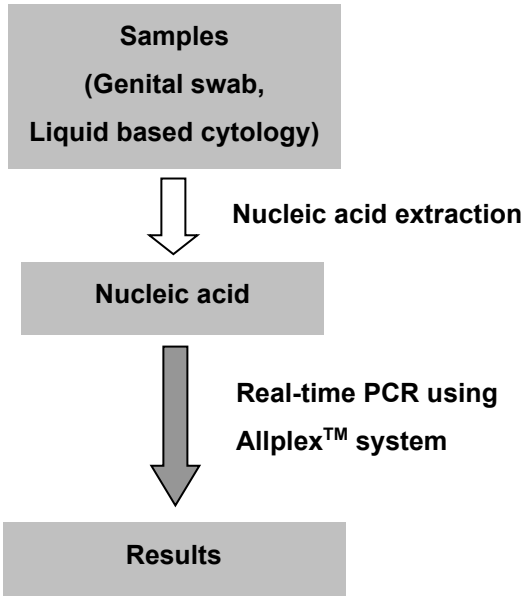
PRINCIPLES AND PROCEDURE OVERVIEW

1. Principles

Allplex™ Vaginitis Screening Assay exhibits Seegene's proprietary MuDT™ technology, which allows to provide multi-Ct (threshold cycle) values in a single fluorescence channel without melt curve analysis on real-time PCR instruments.
Allplex™ Vaginitis Screening Assay is a multiplex real-time PCR assay that permits simultaneous amplification and detection of target nucleic acids of *Lactobacillus* spp. (Lacto), *Gardnerella vaginalis* (GV), *Atopobium vaginae* (AV), *Mobiluncus* spp. (Mob), *Candida albicans* (CA), Candida others (CO), *Trichomonas vaginalis* (TV) and Internal Control. The presence of specific gene sequence in the reaction is reported as a Ct value and Qt value through Seegene Viewer analysis software.
An endogenous human gene is used as Internal Control (IC) to monitor the whole process of sample collection, nucleic acid extraction and to check for any possible PCR inhibition.

To prevent amplification product acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ Vaginitis Screening Assay. The UDG-dUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG excises uracil residues from DNA by cleaving the N-glycosylic bond.

2. Procedure Overview



BACKGROUND INFORMATION

Most women will have a vaginal infection, characterized by discharge, itching, or odor, during their lifetime. Obtaining a medical history alone has been shown to be insufficient for accurate diagnosis of vaginitis and can lead to the inappropriate administration of medication. Therefore, a careful history, examination, and laboratory testing to determine the etiology of vaginal symptoms are warranted. The three most common conditions diagnosed among women with vaginal symptoms presenting in the primary care setting were bacterial vaginosis (22% to 50%), vulvovaginal candidiasis (17% to 39%), and trichomoniasis (4% to 35%). In some cases, the etiology may be mixed, and there may be more than one disease present; in approximately 30% of symptomatic women, no etiologic agent is identified.

Bacterial vaginosis (BV) is a polymicrobial clinical syndrome resulting from replacement of the normal hydrogen peroxide producing *Lactobacillus* sp. in the vagina with high concentrations of anaerobic bacteria (e.g. *Prevotella* sp. and *Mobiluncus* sp.), *G. vaginalis*, Ureaplasma, Mycoplasma, and numerous fastidious or uncultivated anaerobes. BV can be diagnosed by the use of clinical criteria (i.e., Amsel's Diagnostic Criteria) or Gram stain (considered the gold standard laboratory method for diagnosing BV). PCR has been used in research settings for the detection of variety of organisms associated with BV. Detection of specific organisms might be predictive of BV by PCR. Additional validation is needed before these tests can be recommended to diagnose BV.

Vulvovaginal candidiasis (VVC) is caused by *C. albicans* but can occasionally be caused by other *Candida* sp. or yeast. An estimated 75% of women will have at least one episode of VVC, and 40% to 45% will have two or more episodes. On the basis of clinical presentation, microbiology, host factors, and response to therapy, VVC can be classified as either uncomplicated or complicated. Approximately 10% to 20% of women will have complicated VVC, requiring special diagnostic and therapeutic considerations. Conventional antimycotic therapies are not as effective against these nonalbicans species as against *C. albicans*.

Trichomoniasis is a very common sexually transmitted disease (STD). It is caused by infection with a protozoan parasite called *Trichomonas vaginalis*. About 70% of infected people do not have any signs or symptoms. Wet-mount microscopy of a vaginal swab often reveals white blood cells and rapidly motile trichomonads. However, detection of trichomonads by microscopy has a sensitivity of only 60% to 75%, whereas polymerase chain reaction (PCR) can detect *T. vaginalis* with a sensitivity of 85% to 100%.

REAGENTS

The reagents contained in one kit are sufficient for 100 reactions.

Order information (REF SD9750X)

Allplex™ Vaginitis Screening Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	VS MOM	500 µL	MuDT Oligo Mix (MOM): - Amplification and detection reagent
PREMIX	EM1	500 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	VS PC	80 µL	Positive Control (PC): - Mixture of pathogen and IC clones
SD	VS SD1	80 µL	Standard DNA for Quantitation ($1X10^7$ copies/rxn) - Mixture of 3 pathogen clones (Lacto, GV, AV)
SD	VS SD2	80 µL	Standard DNA for Quantitation ($1X10^5$ copies/rxn) - Mixture of 3 pathogen clones (Lacto, GV, AV)
SD	VS SD3	80 µL	Standard DNA for Quantitation ($1X10^3$ copies/rxn) - Mixture of 3 pathogen clones (Lacto, GV, AV)
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

Accessory product – analysis software
Seegene Viewer*

* The analysis software is provided by Seegene or regional manager. Please use Seegene Viewer beyond V3.

The reagents contained in one kit are sufficient for 25 reactions.

Order information (REF SD10321Z)

AllplexTM Vaginitis Screening Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	VS MOM	125 µL	MuDT Oligo Mix (MOM): - Amplification and detection reagent
PREMIX	EM1	125 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	VS PC	80 µL	Positive Control (PC): - Mixture of pathogen and IC clones
SD	VS SD1	80 µL	Standard DNA for Quantitation ($1X10^7$ copies/rxn) - Mixture of 3 pathogen clones (Lacto, GV, AV)
SD	VS SD2	80 µL	Standard DNA for Quantitation ($1X10^5$ copies/rxn) - Mixture of 3 pathogen clones (Lacto, GV, AV)
SD	VS SD3	80 µL	Standard DNA for Quantitation ($1X10^3$ copies/rxn) - Mixture of 3 pathogen clones (Lacto, GV, AV)
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

Accessory product – analysis software
Seegene Viewer*

* The analysis software is provided by Seegene or regional manager. Please use Seegene Viewer beyond V3.

STORAGE AND HANDLING

All components of Allplex™ Vaginitis Screening Assay should be stored at ≤ -20°C. All components are stable under recommended storage conditions until the expiry date stated on the label. The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, reagents should be stored in aliquots.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Ice maker
- Desktop centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection System (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)
- Optical Flat 8-Cap Strips (Cat No. TCS0803, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Cat. No. HSP9655, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Cat. No. HSP9955, Bio-Rad)
- AIOS (Cat. No. SG72100, Seegene)
- Pierceable cap (Cat. No. 922119, SPL) (for AIOS use only)
- Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)*
- PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)*
- Saline solution
- Clean bench

* Make sure to use the heat seal and the plate sealer listed above together.

PROTOCOL

1. Specimen Collection, Storage, and Transport

Note: All samples should be treated as potentially infectious materials. Only those sample materials are permitted, which are collected, transported and stored attending strictly to the following rules and instructions.

Genital swab
Liquid based cytology

Note: To ensure high quality of samples, samples should be transported as fast as possible at indicated temperature.

A. Specimen Collection

Genital swab

For the collection of genital swabs, please use following material:

- Genital swabs can be collected and transported in 1~3 mL of following mediums :
  - ENAT PM 2ML REGULAR APPLICATOR (Copan)
  - UTM with Flocked Swabs (Copan)
  - Swab Specimen Collection Kit (Qiagen Corporation)
- Leave the swab in the transport medium. Close and label the sample container. Strictly adhere to given instructions for storage and transport.
- Please follow a recommended protocol for collection of columnar and squamous epithelium cells after removal of the cervical mucus.

Liquid based cytology

- Use liquid based cytology media ThinPrep® from HOLOGIC® Inc. and SurePath™ from BD.
- Follow the manufacturer's instructions for collection of cervical cell specimens into ThinPrep® and SurePath™ media.

B. Specimen Storage & Transport

Specimen	Storage & Transport		Note
	Temp.	Duration*	
Genital swab	2~8℃	1 week	- Performance may be affected by prolonged storage of specimens. - Specimens should also adhere to local and national instructions for transport of pathogenic material.
ThinPrep® medium	2~8℃	90 days	
SurePath™ medium	2~8℃	2 weeks	

* Duration: The time period from the specimen collection to the final test (includes transport and storage of specimens in prior to test)

2. Nucleic Acid Extraction

A. Pre-treatment of specimens

Genital swab

- Genital swab is used without pre-treatment.

Liquid based cytology

- Equilibrate samples to room temperature (19~25℃).
- Centrifuge 1 mL of liquid based cervical cytology for 15 minutes at 15,000 x *g* (13,000 rpm).
- Discard supernatant and resuspend the pellet in the recommended volume of Saline solution (See Recommended Vol. of 2-B) by vortexing thoroughly to redissolve and disperse the sample.

Note: Process pre-treatment step using lysis buffer in extraction kit not saline solution if the samples are collected in SurePath™ medium and would be analyzed with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet.

Note: ThinPrep® media can be processed without pre-treatment when using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet.

- Follow the manufacturer's protocol.

B. Manual Nucleic Acid Extraction Kits

Note: Please use the recommended specimen and elution volumes as indicated below. For others, refer to the manufacturer's manual.

Extraction Kit	Manufacturer	Cat. No.	Recommended Vol.
QIAamp® DSP DNA Mini Kit*	QIAGEN	61304	Specimen: 200 μL Elution: 50 μL
QIAamp® DNA Mini Kit*	QIAGEN	51304	Specimen: 200 μL Elution: 50 μL
Ribo_spin vRD** (Viral RNA/DNA Extraction Kit)	GeneAll	302-150 SG1701***	Specimen: 200 μL Elution: 50 μL

* Process lysis step using 180 μL of ATL buffer instead of AL buffer in case of SurePath™ media.

** Ribo_spin vRD kit is not compatible with SurePath™ media.

*** Please use catalog numbers shown above to purchase products from Seegene Inc.

C. Automated Extraction System

Note: Please use the recommended specimen and elution volumes as indicated below. For others, refer to the manufacturer's manual.

C-1. NucliSENS® easyMAG®

Note: See NucliSENS® easyMAG® operation manual.

Automated Extraction System	Manufacturer	REF	Recommended Vol.
NucliSENS® easyMAG®	bioMérieux	200111	Specimen: 200 μL Elution: 100 μL

C-2. Microlab NIMBUS IVD

Note: See Microlab NIMBUS IVD operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab NIMBUS IVD	Hamilton	65415-02*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 μL Elution: 100 μL

*Please use catalog numbers shown above to purchase products from Seegene Inc.

C-3. Microlab STARlet IVD

Note: See Microlab STARlet IVD operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab STARlet IVD	Hamilton	173000-075*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 μL Elution: 100 μL

*Please use catalog numbers shown above to purchase products from Seegene Inc.

C-4. Seegene NIMBUS

Note: See Seegene NIMBUS operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene NIMBUS	Seegene	65415-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 μL Elution: 100 μL

C-5. Seegene STARlet

Note: See Seegene STARlet operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene STARlet	Seegene	67930-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 μL Elution: 100 μL

Option: Automated Linkage Structure (See AIOS operation manual)

Automated Linkage Structure	Manufacturer	Cat. No.
AIOS	Seegene	SG72100

Note: Replace the cap of the Positive Control (PC) and Standard DNA (SD) with a pierceable cap. After finishing the operation, replace the cap of the Positive Control (PC) and Standard DNA (SD) with the original cap.

Note: The pierceable cap is a single-use product and must be disposed of after one use.

Note: If used with AIOS, this product can be used for maximum 3 separate runs.

3. Preparation for Real-time PCR

Note: Correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).

Note: Aerosol resistant filter tips and tight gloves must be used when preparing PCR reactions. Use extreme care to prevent cross-contamination.

Note: Completely thaw all reagents on ice.

Note: Spin down the reagent tubes to collect residual drops inside of the cap.

Note: The steps A~D are automatically processed on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet. Refer to each operation manual.

A. Prepare PCR Mastermix.

5 μL	VS MOM
5 μL	EM1
5 μL	RNase free water
15 μL	Total volume of PCR Mastermix

Note: Calculate the total amount of each reagent needed based on the number of reactions (samples + controls + standard DNAs).

B. Mix by quick vortexing, and briefly centrifuge.

C. Aliquot 15 μL of PCR Mastermix into PCR tubes.

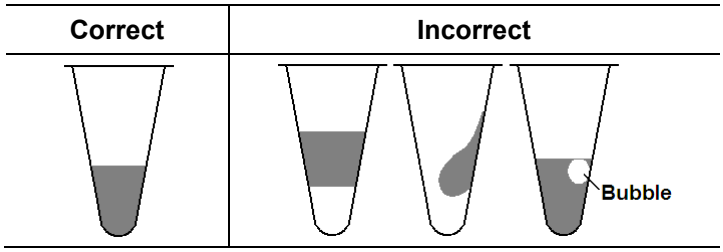
D. Add 5 μL of each sample's nucleic acids into the tube containing PCR Mastermix.

15 μL	PCR Mastermix
5 μL	Sample's nucleic acid
20 μL	Total volume of reaction

E. Close and spin down the PCR tubes.

F. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher rpm for a longer time.

Note: It is recommended to spin down the PCR tubes before PCR to eliminate air bubbles and collect all residual liquids at the bottom of tubes.



Note: Use a new sterile pipette tip for each sample.

Note: For **Negative Control (NC)**, use 5 μL of RNase-free Water instead of sample's nucleic acid.

Note: For **Positive Control (PC)**, use 5 μL of VS PC instead of sample's nucleic acid.

Note: For **Standard DNA (SD)**, use 5 μL of VS SD1, VS SD2 and VS SD3 instead of sample's nucleic acid.

Note: Location of Negative Control, Positive Control and Standard DNA are set in the Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene NIMBUS in accordance with the number of samples.

Note: Please be careful not to cross-contaminate the PCR Mastermix and samples with Positive Control and the Standard DNA.

Note: Do not label the reaction tube on its cap. Fluorescence is detected from the top of each reaction tube.

REAL-TIME PCR INSTRUMENT SET UP AND RESULTS ANALYSIS

1. CFX96™ Real-time PCR Detection System (CFX Manager™ Software-IVD v1.6)

1.1. Real-time PCR Instrument set up

Note: CFX96™ Real-time PCR Detection System (Bio-Rad) experiment setup can be divided into three steps: Protocol Setup, Plate Setup, and Start run.

A. Protocol Setup

1) In the main menu, select **File** → **New** → **Protocol** to open **Protocol Editor**.

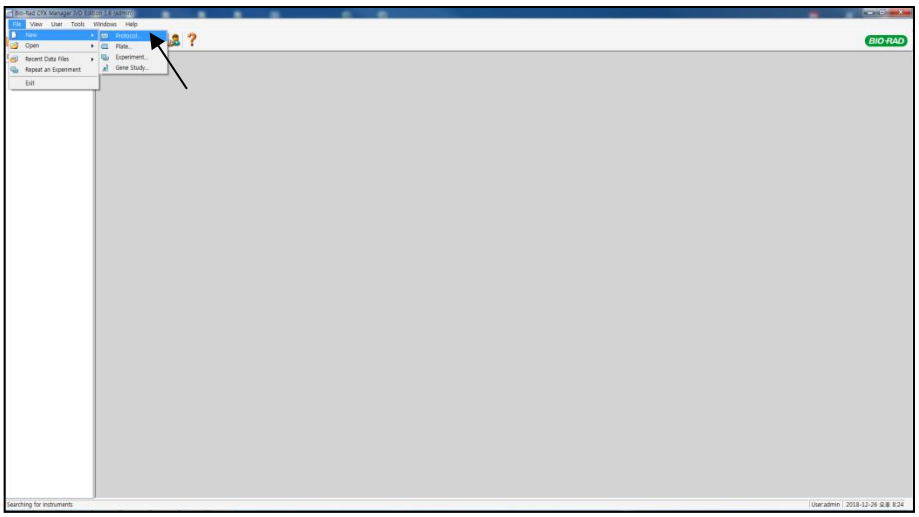


Fig. 1. **Protocol Setup**.

2) In **Protocol Editor**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	50°C	4 min
2		95°C	15 min
3	5	95°C	30 sec
4		60°C	1 min
5		72°C	30 sec
6	GOTO 3, 4 more times		
7	40	95°C	10 sec
8*		60°C	1 min
9*		72°C	10 sec
10	GOTO 7, 39 more times		

Note*: Plate Read at Step 8 and 9. Fluorescence is detected at 60°C and 72°C.

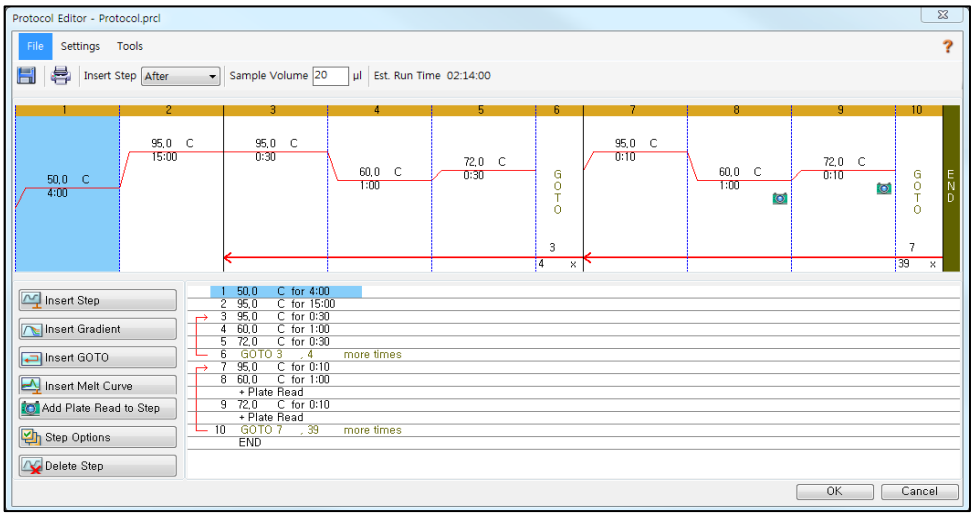


Fig. 2. **Protocol Editor**

3) Click the box next to **Sample Volume** to directly input 20 µL.

4) Click **OK** and save the protocol to open the **Experiment Setup** window.

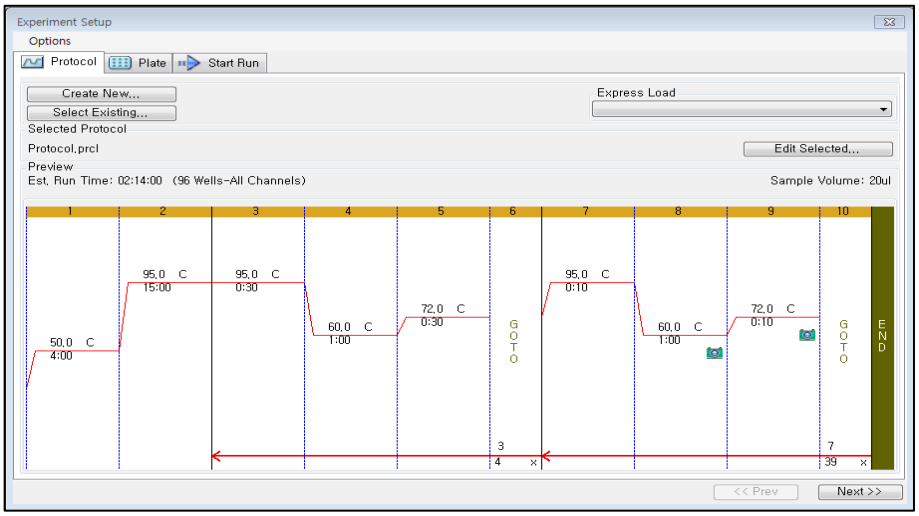


Fig. 3. **Experiment Setup: Protocol**

B. Plate Setup

1) From **Plate** tab in **Experiment Setup**, click **Create New** to open **Plate Editor** window.

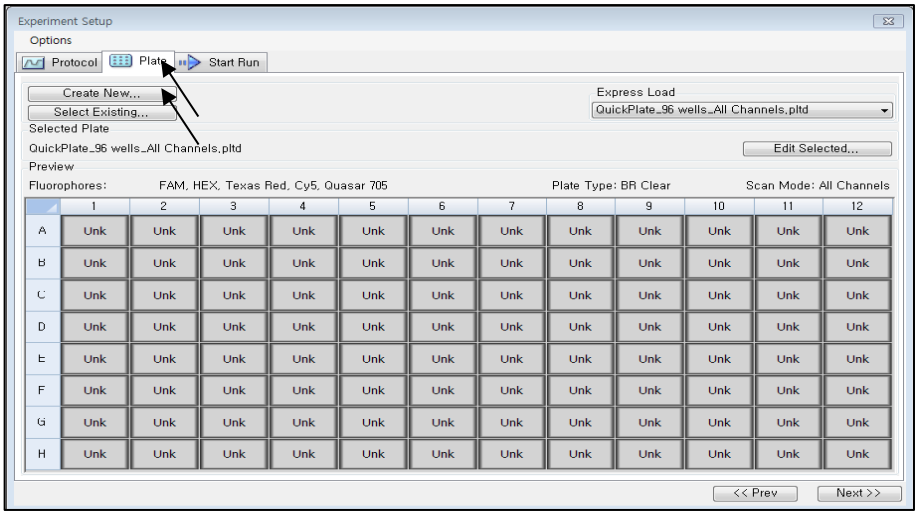


Fig. 4. **Plate Editor**.

2) Click **Select Fluorophores** to indicate the fluorophores (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**) that will be used and click **OK**.

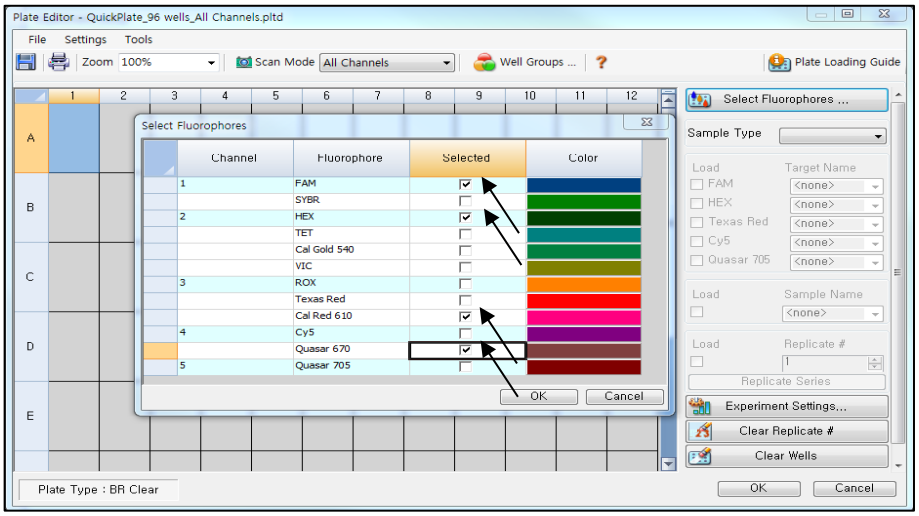


Fig. 5. **Select Fluorophores** (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**)

3) Creating a Standard Curve

- Select the wells loaded with Sample Type/Standard, designate a specific fluorophore (FAM, HEX, Cal Red 610, and Quasar 670) and then click the Load checkbox (Fig. 6).
- Select the wells in the plate diagram and click Replicate Series. Then Replicate Series editing window will be opened (Fig. 7).
- Select the wells that have been assigned consecutive replicate numbers and click Dilution Series. Then input Dilution Series window as shown in Fig. 8.

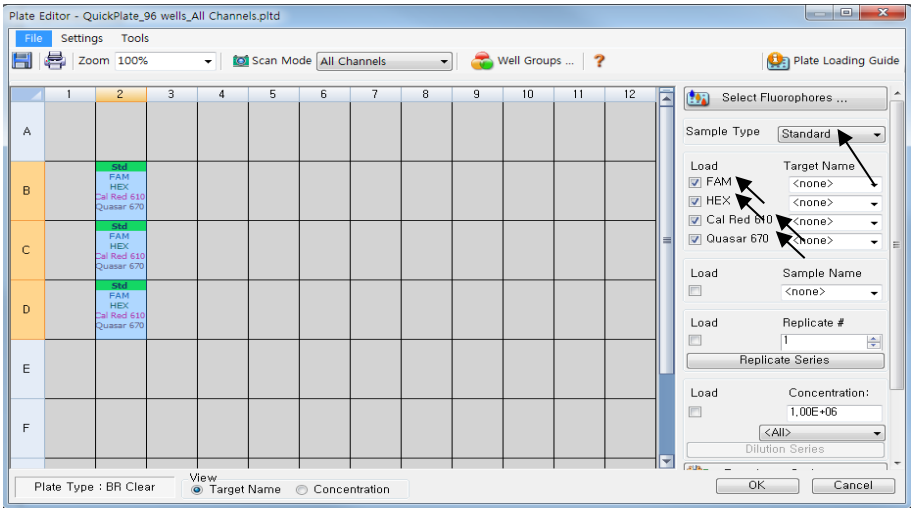


Fig. 6. **Creating a Standard Curve (1)**

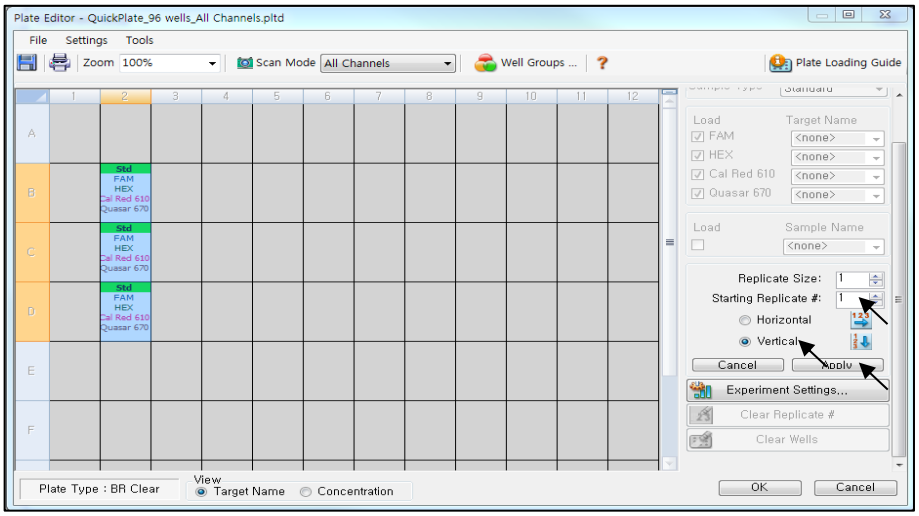
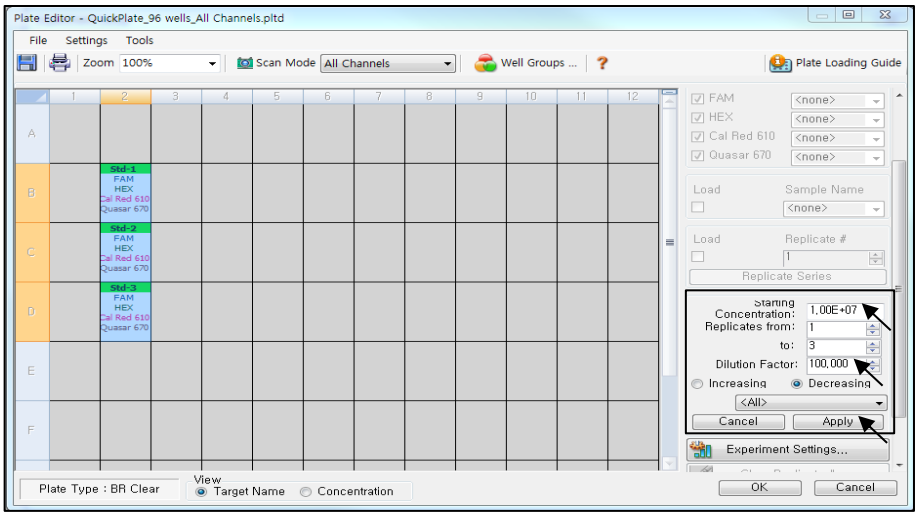


Fig. 7. **Creating a Standard Curve (2)**



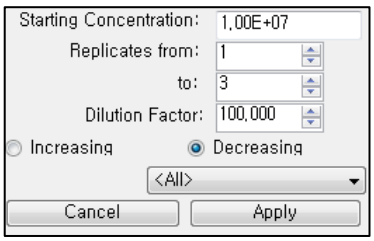


Fig. 8. **Creating a Standard Curve (3)**

4) Select the wells where the PCR tube will be placed and select its sample type from the **Sample Type** drop-down menu.

- **Unknown**: Clinical samples
- **Negative Control**
- **Positive Control**
- **Standard DNA**

5) Click on the appropriate checkboxes (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**) to specify the fluorophores to be detected in the selected wells.

6) Type **Sample Name** and press enter key.

7) In **Settings** of the **Plate Editor** main menu, choose the **Plate Size (96 wells)** and **Plate Type (BR White)**.

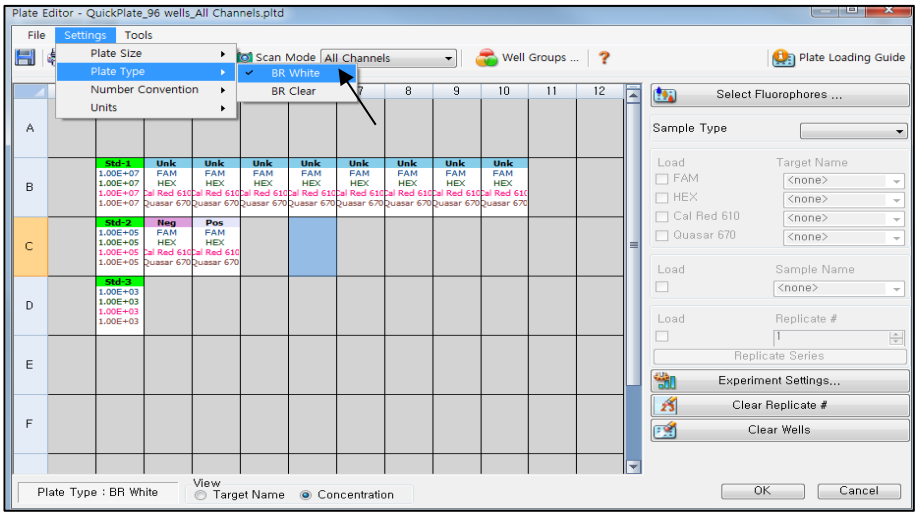


Fig. 9. **Plate Setup**

8) Click **OK** to save the new plate.

9) You will be returned to the **Experiment Setup** window.

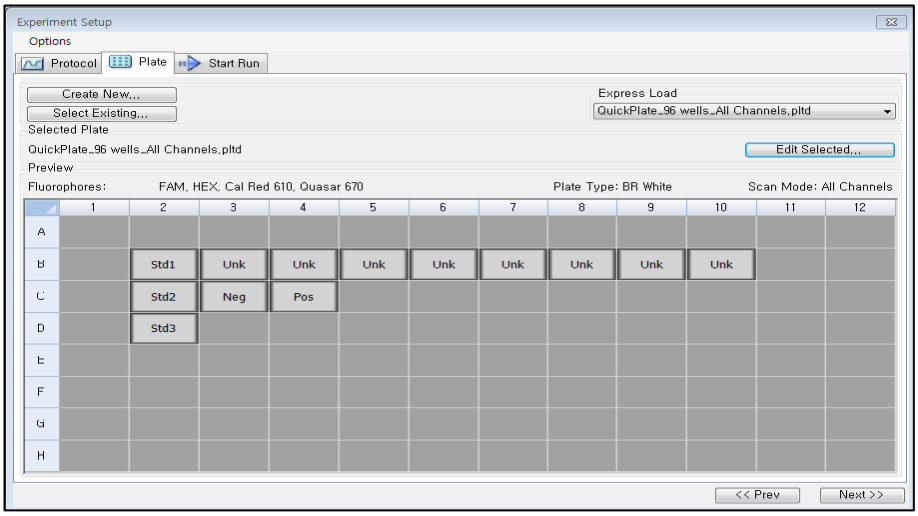


Fig. 10. **Experiment Setup: Plate**

10) Click **Next** to start run.

C. Start Run

1) From **Start Run** tab in **Experiment Setup**, click **Close Lid** to close the instrument lid.

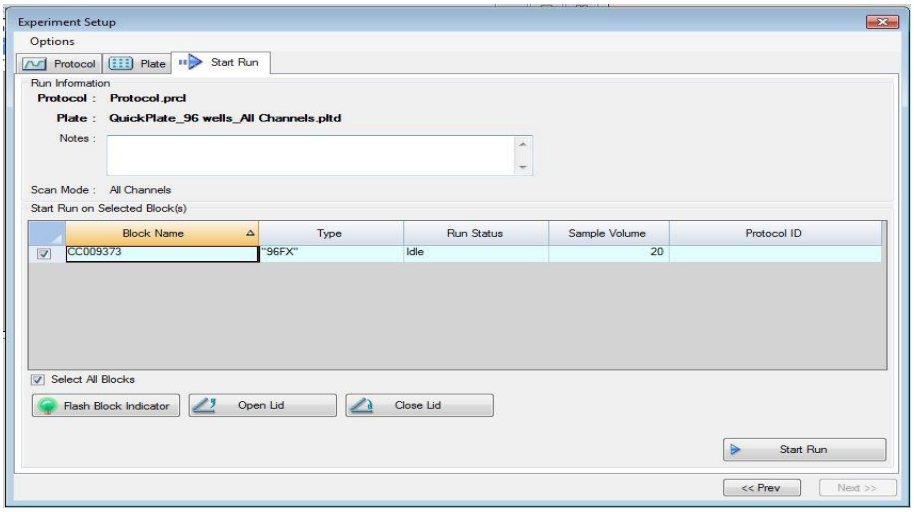


Fig. 11. **Close Lid.**

2) Click **Start Run**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **SAVE**, and the run will start.

1.2. Data Analysis

A. Create folders for data export

1) To save data for all of amplification curve detection step from the result file, create one folder.
2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX Manager

1) After the test, click the Quantitation tab to confirm the amplification curve results.

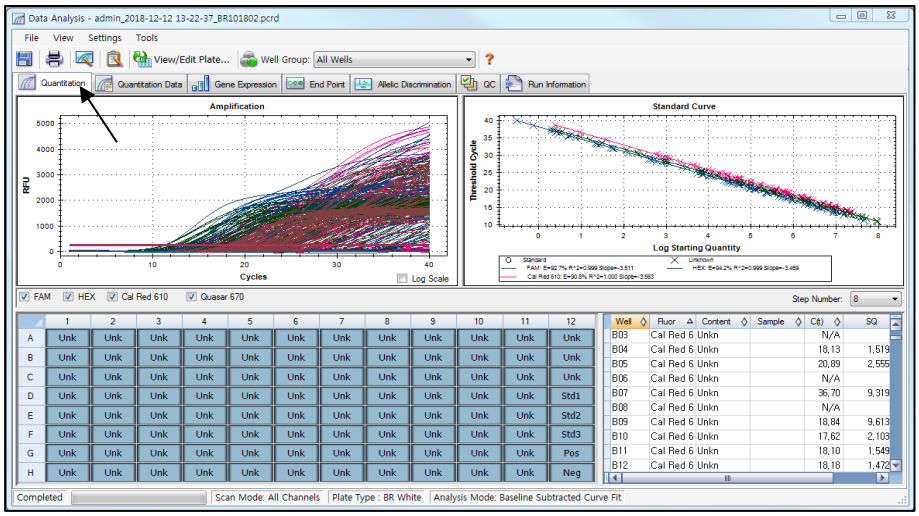


Fig. 12. **Amplification curve results**

2) Select **No Baseline Subtraction** from Analysis Mode of Settings menu.

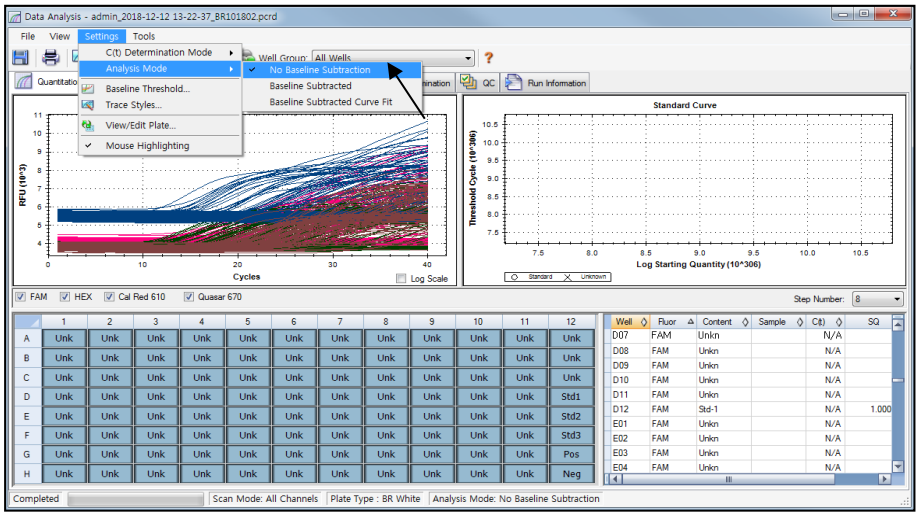


Fig. 13. **No Baseline Subtraction**

3) Select **Seegene Export** from Tools menu.

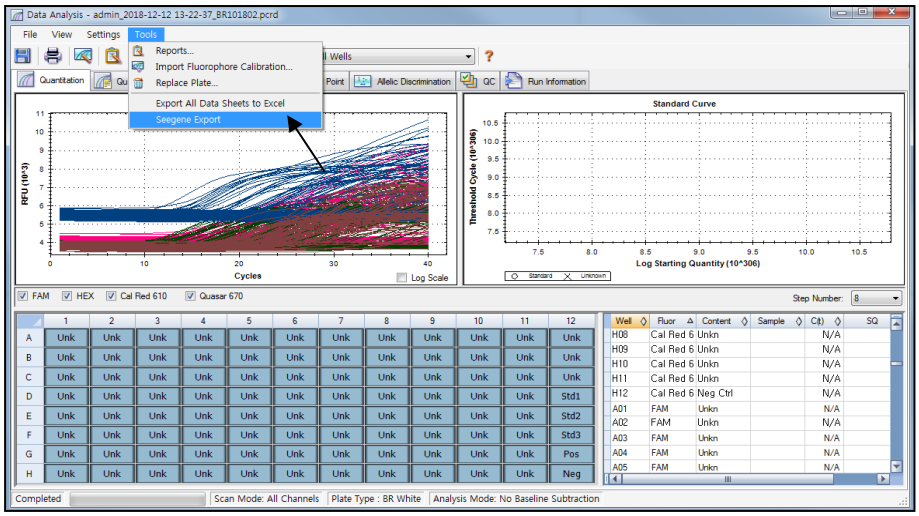


Fig. 14. **Seegene Export**

4) Choose a location to save data and click **OK**.

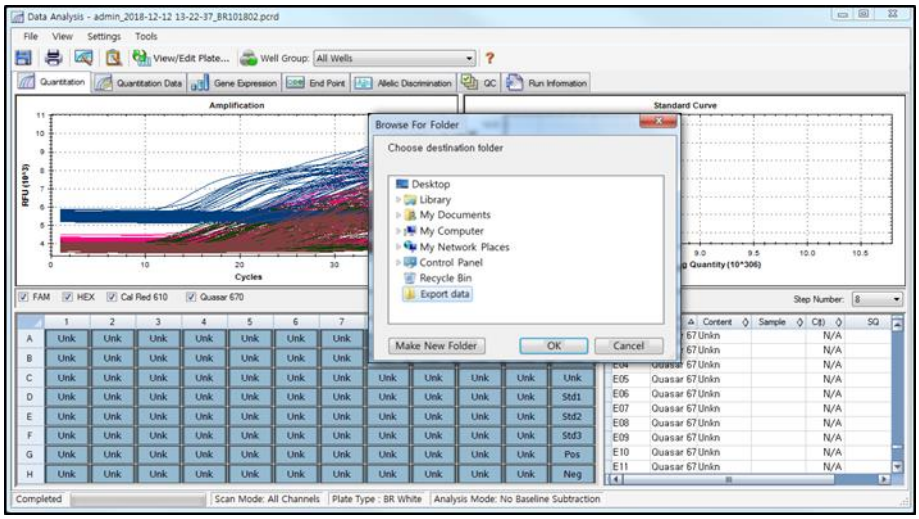


Fig. 15. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program and click **Option** to select **CFX96** in the **Instrument**.

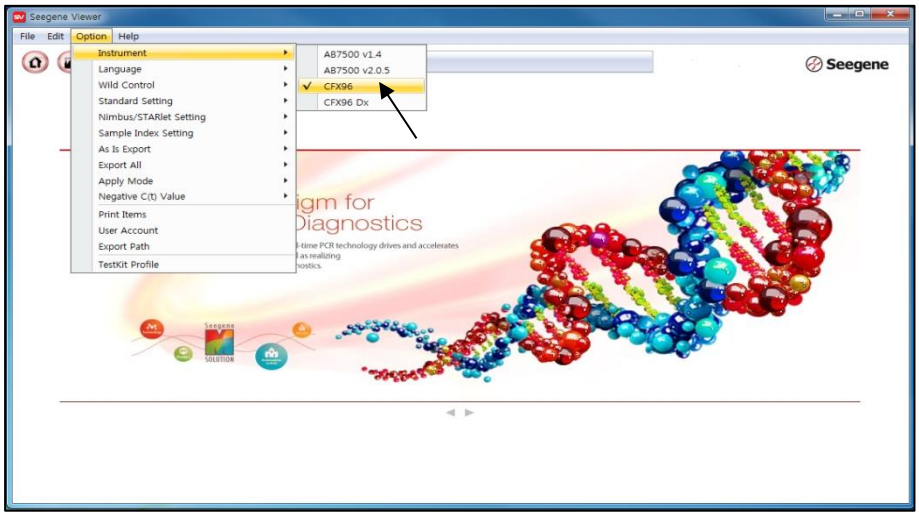


Fig. 16. **Seegene Viewer**

2) Click **Open** to find the saved file in folder “QuantStep8”, open the result file, and select the test kit from the **PRODUCT** menu.

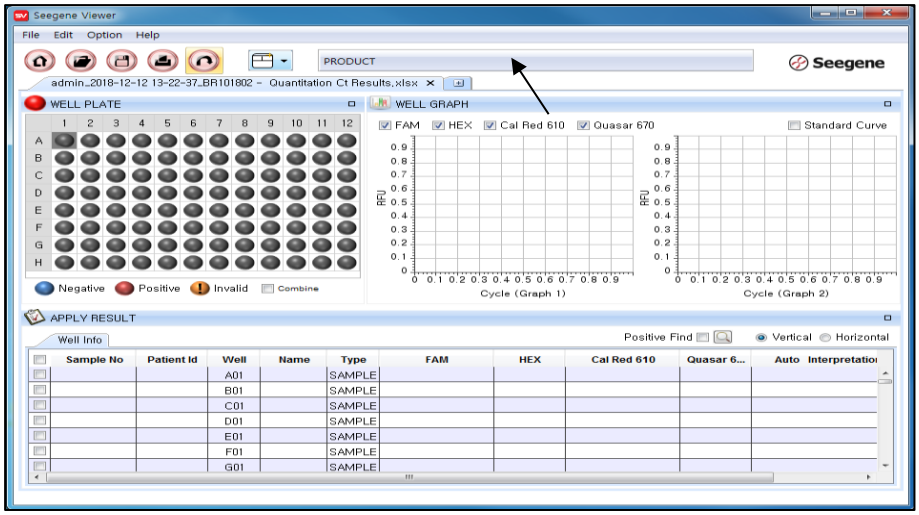


Fig. 17. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

Fig. 18. **Test result on Seegene Viewer**

4) Validity Criteria of Control Results

a. Valid Assay Run

To confirm the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

Control	Seegene Viewer Result								
	FAM		HEX		Cal Red 610		Quasar670		Auto Interpretation
	CA	Lacto	CO	GV	TV	AV	Mob	IC	
	Ct	Qt	Ct	Qt	Ct	Qt	Ct	Ct	
Positive Control	≤ 40	> 0	≤ 40	> 0	≤ 40	> 0	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

b. Invalid Assay Run

In cases of a validity failure, the results should not be interpreted or reported, and the PCR reaction must be repeated.

2. CFX96™ Dx System (CFX Manager™ Dx Software-IVD v3.1)

2.1. Real-time PCR Instrument set up

Note: CFX96™ Dx System (Bio-Rad) experiment setup can be divided into three steps: Protocol Setup, Plate Setup, and Start run.

A. Protocol Setup

1) In the main menu, select **File** → **New** → **Protocol** to open **Protocol Editor**.

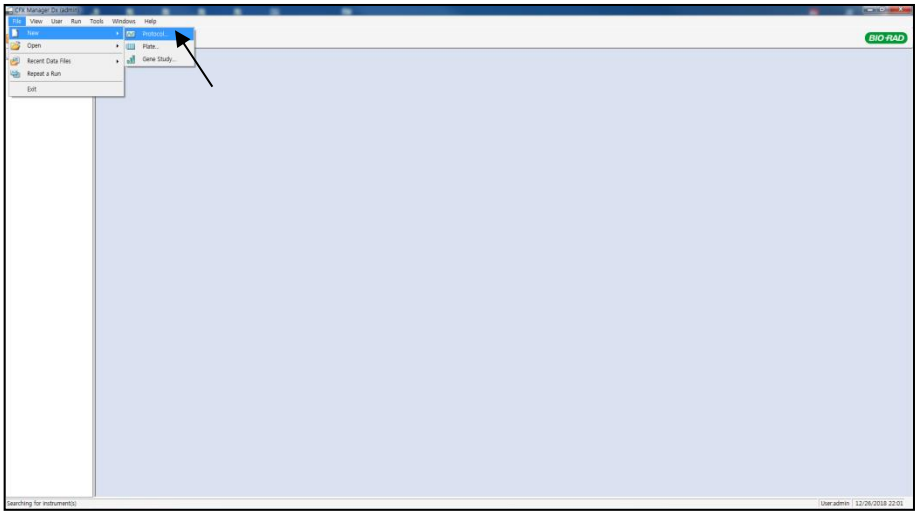


Fig. 1. **Protocol Setup**.

2) In **Protocol Editor**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	50°C	4 min
2		95°C	15 min
3	5	95°C	30 sec
4		60°C	1 min
5		72°C	30 sec
6	GOTO 3, 4 more times		
7	40	95°C	10 sec
8*		60°C	1 min
9*		72°C	10 sec
10	GOTO 7, 39 more times		

Note*: Plate Read at Step 8 and 9. Fluorescence is detected at 60°C and 72°C.

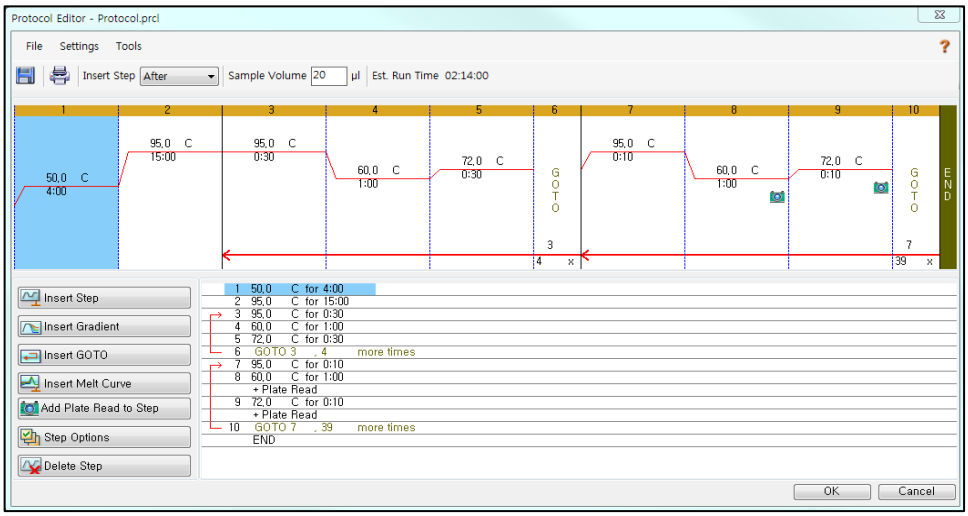


Fig. 2. **Protocol Editor**

3) Click the box next to **Sample Volume** to directly input 20 µL.

4) Click **OK** and save the protocol to open the **Run Setup** window.

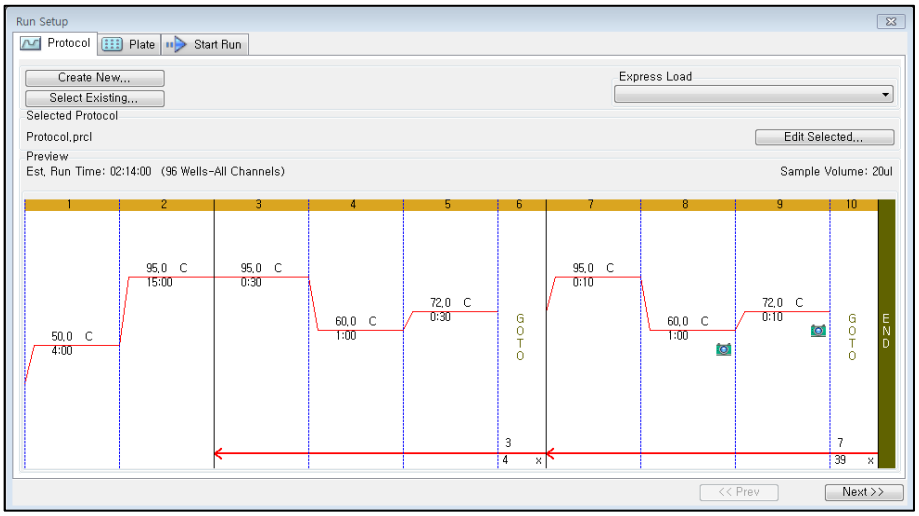


Fig. 3. **Run Setup: Protocol**

B. Plate Setup

1) From **Plate** tab in **Run Setup**, click **Create New** to open **Plate Editor** window.

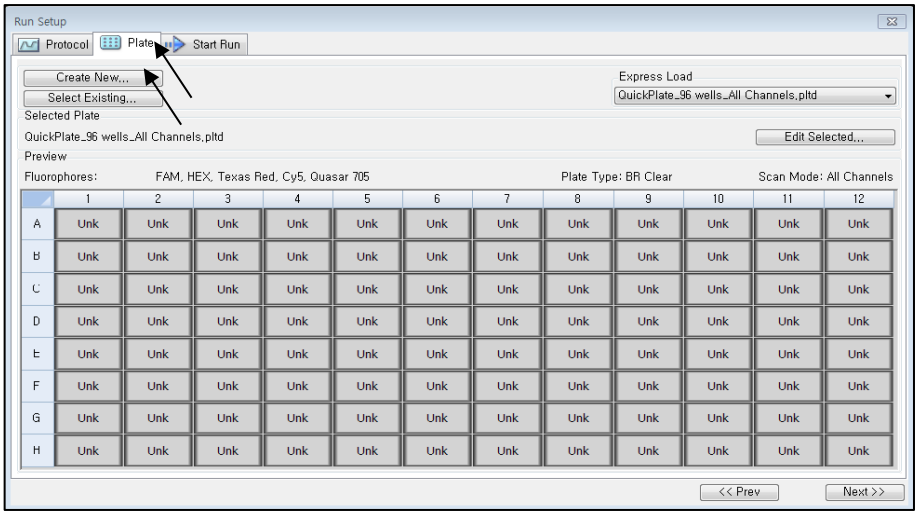


Fig. 4. **Plate Editor**.

2) Click **Select Fluorophores** to indicate the fluorophores (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**) that will be used and click **OK**.

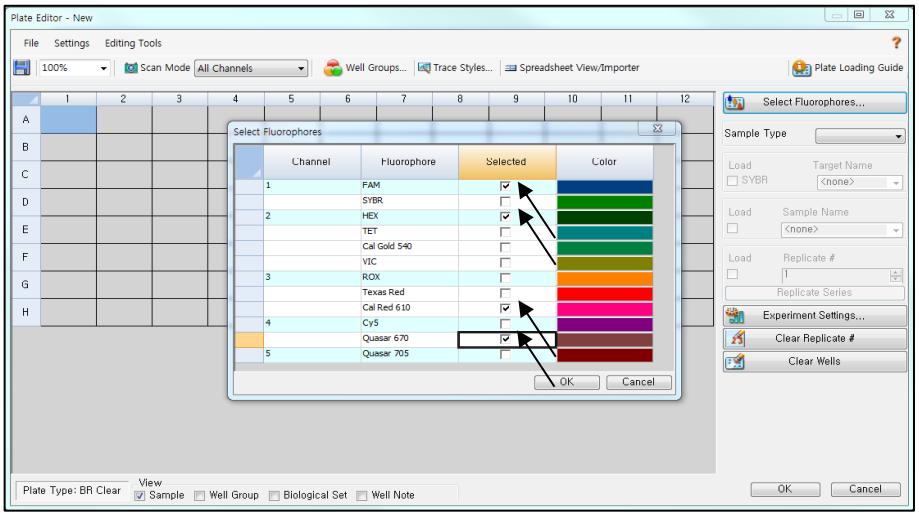


Fig. 5. **Select Fluorophores** (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**)

3) Creating a Standard Curve

- Select the wells loaded with Sample Type/Standard, designate specific fluorophores (FAM, HEX, Cal Red 610, and Quasar 670) and then click the Load checkbox (Fig. 6).
- Select the wells in the plate diagram and click Replicate Series. Then Replicate Series editing window will be opened (Fig. 7).
- Select the wells that have been assigned consecutive replicate numbers and click Dilution Series. Then input Dilution Series window as shown in Fig. 8.

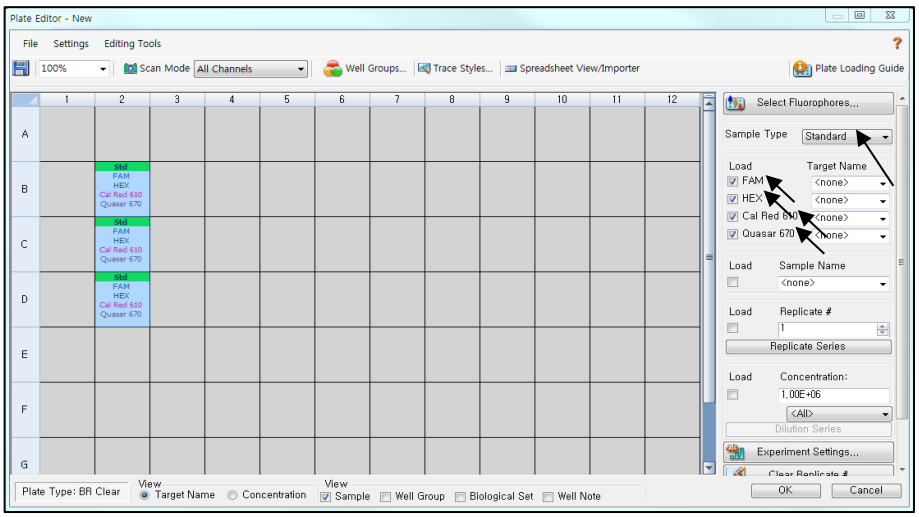


Fig. 6. **Creating a Standard Curve (1)**

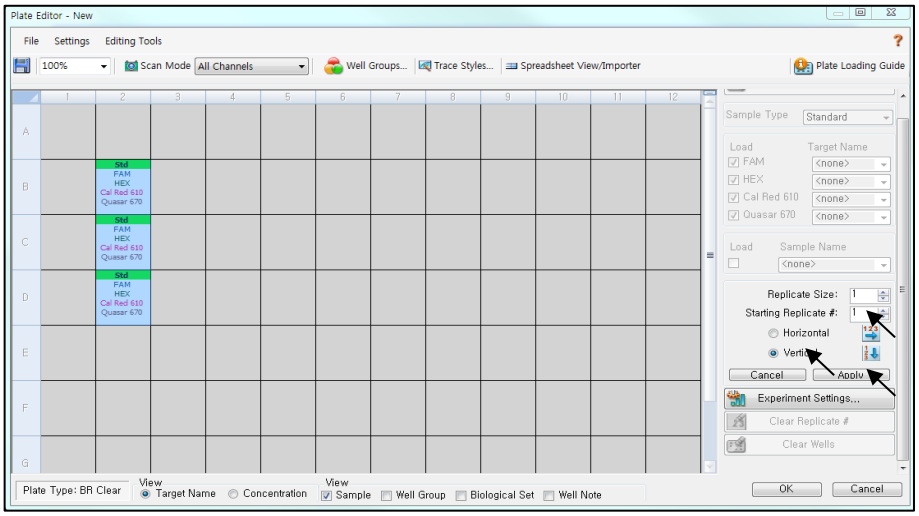
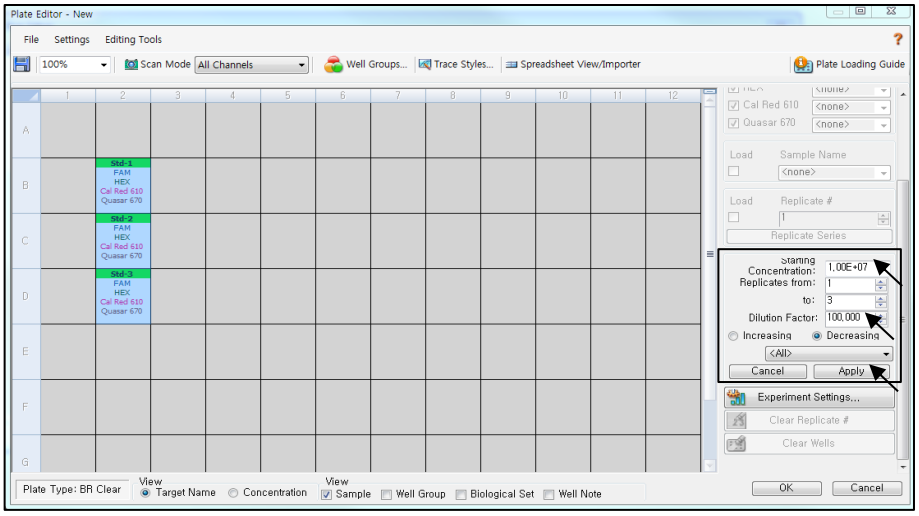


Fig. 7. **Creating a Standard Curve (2)**



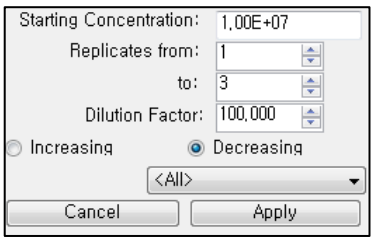


Fig. 8. **Creating a Standard Curve (3)**

4) Select the wells where the PCR tube will be placed and select its sample type from the **Sample Type** drop-down menu.

- **Unknown**: Clinical samples
- **Negative Control**
- **Positive Control**
- **Standard DNA**

5) Click on the appropriate checkboxes (**FAM**, **HEX**, **Cal Red 610** and **Quasar 670**) to specify the fluorophores to be detected in the selected wells.

6) Type **Sample Name** and press enter key.

7) In **Settings** of the **Plate Editor** main menu, choose the **Plate Size (96 wells)** and **Plate Type (BR White)**.

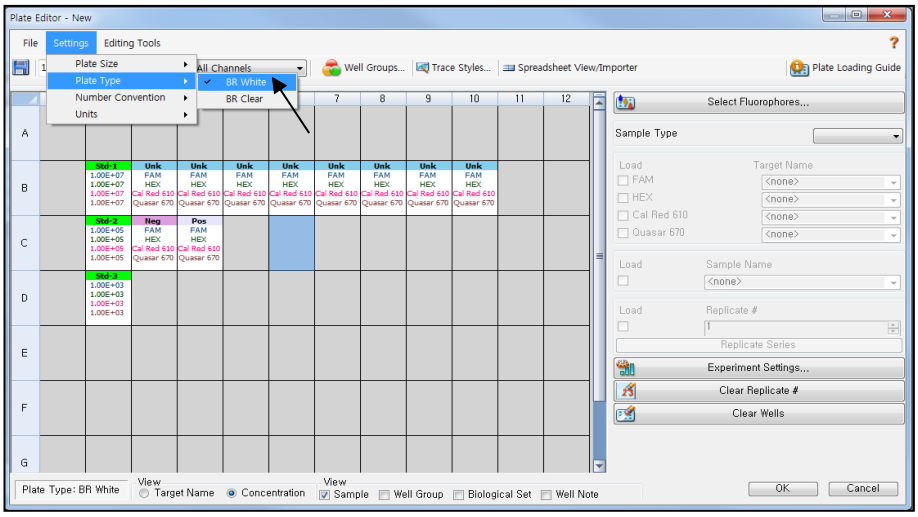


Fig. 9. **Plate Setup**

8) Click **OK** to save the new plate.

9) You will be returned to the **Run Setup** window.

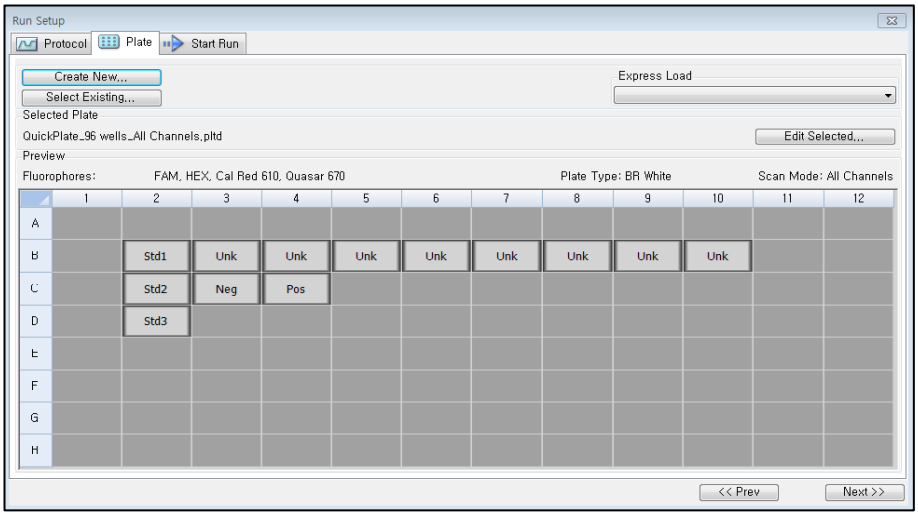


Fig. 10. **Run Setup: Plate**

10) Click **Next** to start run.

C. Start Run

1) From **Start Run** tab in **Run Setup**, click **Close Lid** to close the instrument lid.

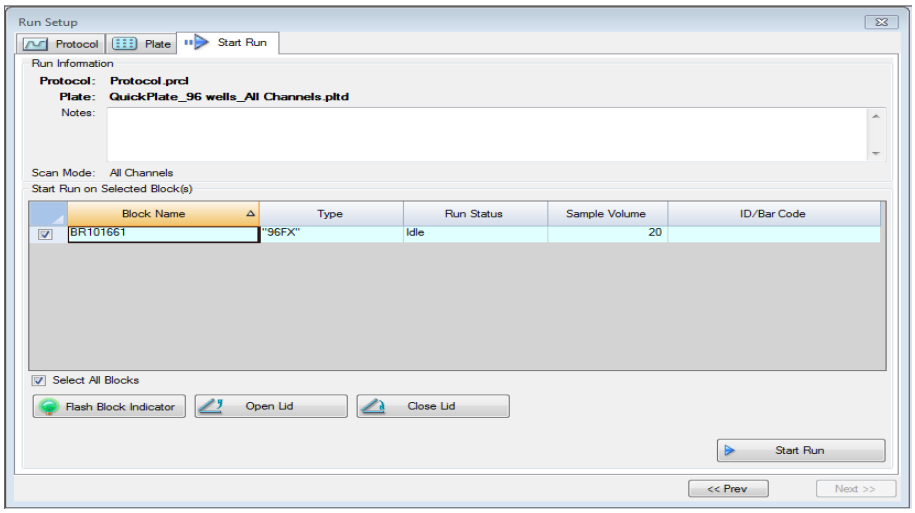


Fig. 11. **Close Lid.**

2) Click **Start Run**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **SAVE**, and the run will start.

1.2. Data Analysis

A. Create folders for data export

1) To save data for all of amplification curve detection step from the result file, create one folder.
2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX Manager™

1) After the test, click the Quantification tab to confirm the amplification curve results.

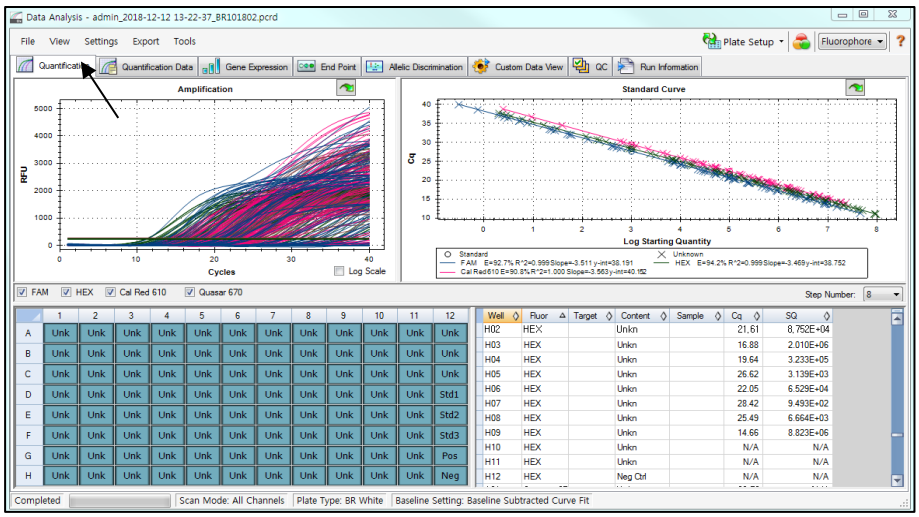


Fig. 12. **Amplification curve results**

2) Select **No Baseline Subtraction** from Baseline Setting of Settings menu.

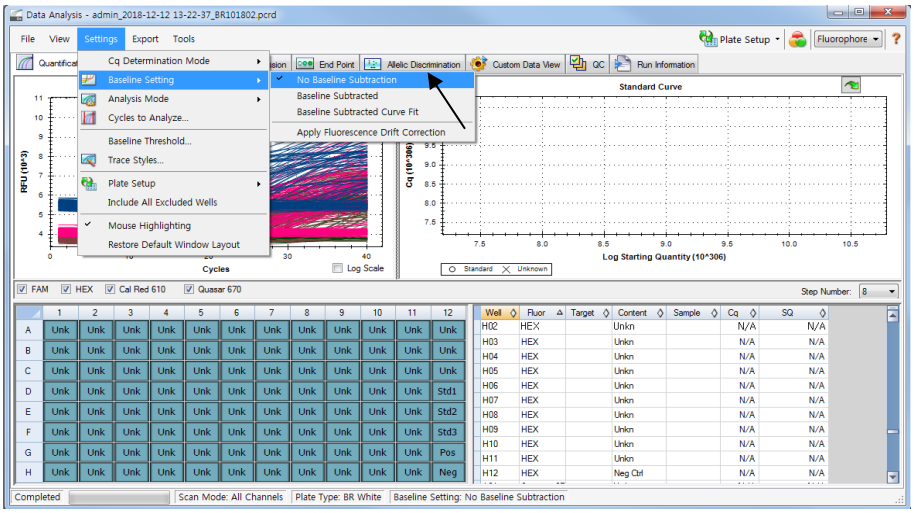


Fig. 13. **No Baseline Subtraction**

3) Select **Seegene Export** from Export menu.

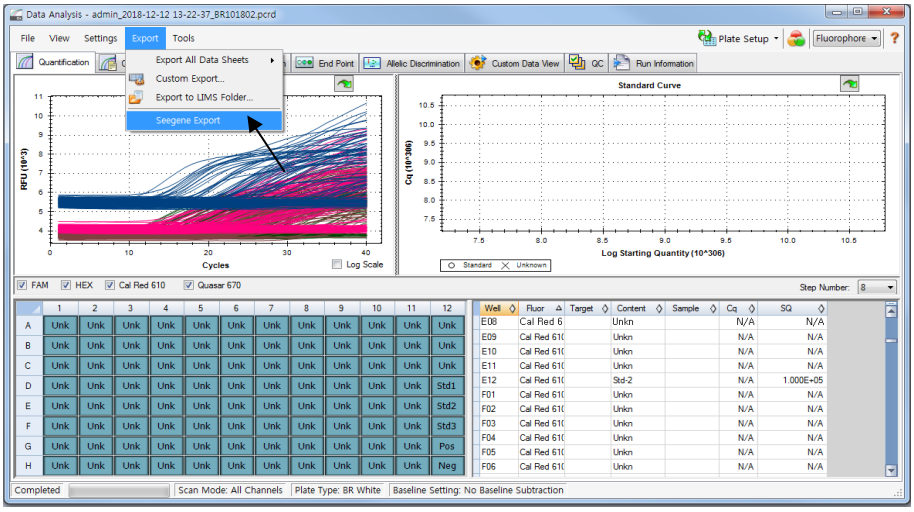


Fig. 14. **Seegene Export**

4) Choose a location to save data and click **OK**.

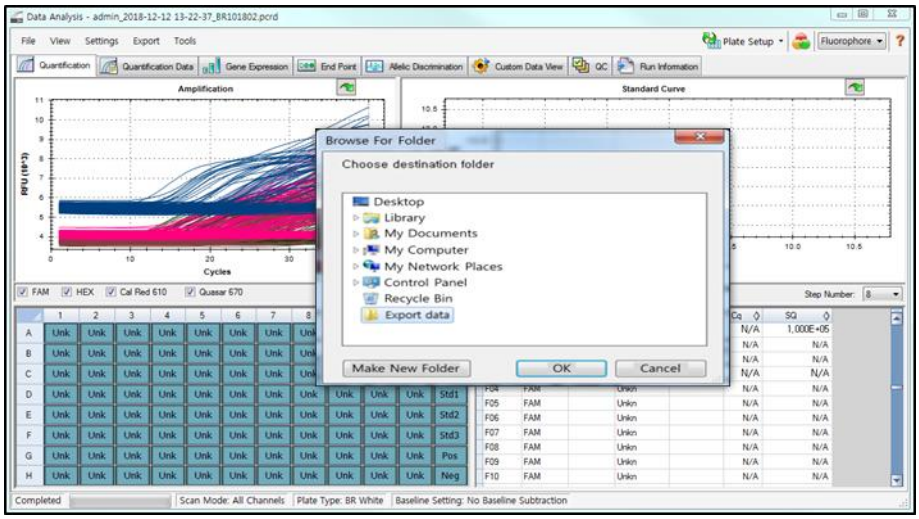


Fig. 15. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program and click **Option** to select **CFX96 Dx** in the **Instrument**.

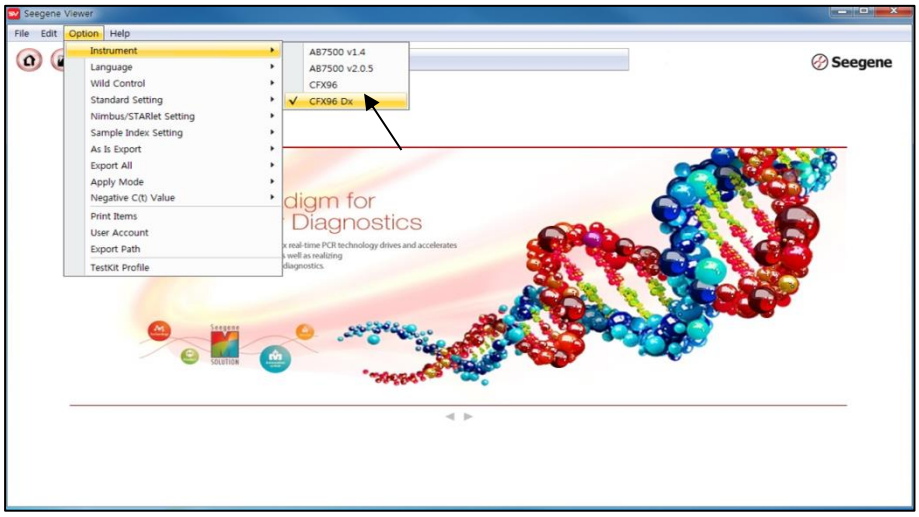


Fig. 16. **Seegene Viewer**

2) Click **Open** to find the saved file in folder “QuantStep8”, open the result file, and select the test kit from the **PRODUCT** menu.

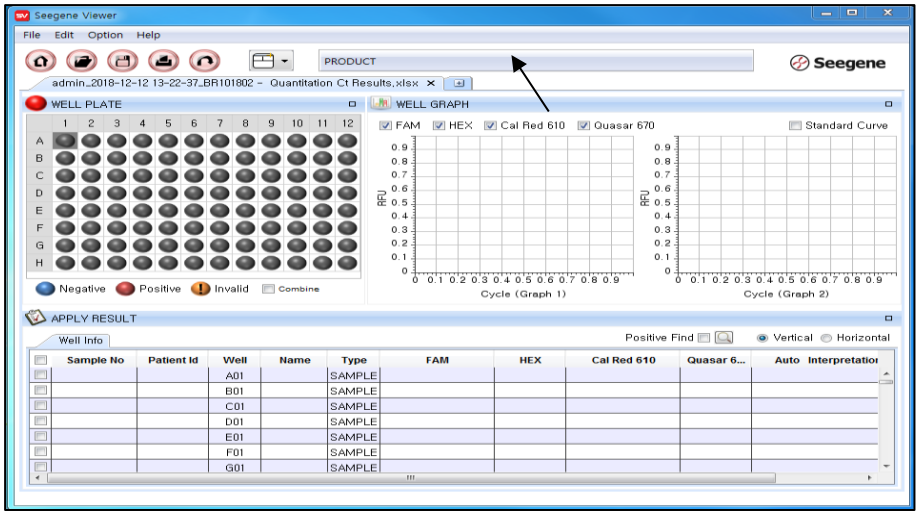


Fig. 17. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

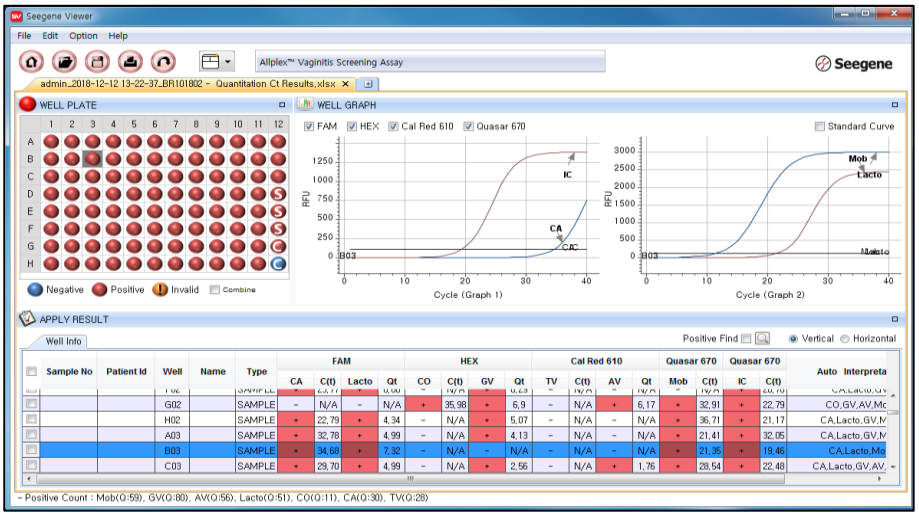


Fig. 18. **Test result on Seegene Viewer**

4) Validity Criteria of Control Results

a. Valid Assay Run

To confirm the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

Control	Seegene Viewer Result								
	FAM		HEX		Cal Red 610		Quasar670		Auto Interpretation
	CA	Lacto	CO	GV	TV	AV	Mob	IC	
	Ct	Qt	Ct	Qt	Ct	Qt	Ct	Ct	
Positive Control	≤ 40	> 0	≤ 40	> 0	≤ 40	> 0	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

b. Invalid Assay Run

In cases of a validity failure, the results should not be interpreted or reported, and the PCR reaction must be repeated.

RESULTS

1. Analytes Information

Fluorophore	Analyte	
	Graph 1	Graph 2
FAM	*Candida albicans* (CA)	*Lactobacillus* spp. * (Lacto)
HEX	*Candida* others** (CO)	*Gardnerella vaginalis* (GV)
Cal Red 610	*Trichomonas vaginalis* (TV)	*Atopobium vaginae* (AV)
Quasar 670	Internal Control (IC)	*Mobiluncus* spp.*** (Mob)

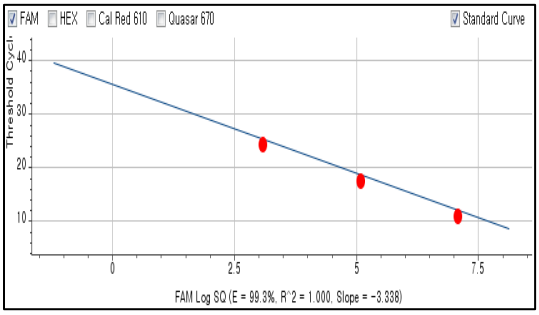
* *Lactobacillus crispatus, Lactobacillus gasseri* and *Lactobacillus jensenii*

** *Candida krusei, Candida glabrata, Candida dubliniensis, Candida parapsilosis, Candida tropicalis* and *Candida lusitaniae*

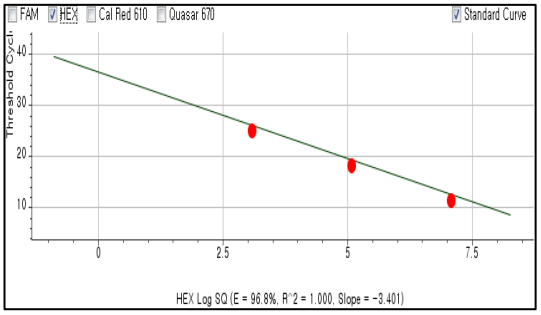
*** *Mobiluncus mulieris* and *Mobiluncus curtisii*

2. Standard Curve Information

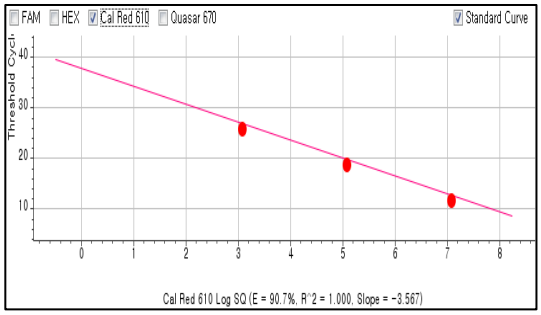
***Lactobacillus* spp.**



Gardnerella vaginalis



Atopobium vaginae



Analyte	R^2
***Lactobacillus* spp.**	0.980 ~ 1.000
Gardnerella vaginalis	0.980 ~ 1.000
Atopobium vaginae	0.980 ~ 1.000

3. Interpretation of Results

3.1. Auto Interpretation

Analyte	Ct[1] value	Qt[2] value	Result
CA, CO, TV, Mob	≤ 40	-	Detected (+)
	N/A	-	Not detected (-)
Lacto, GV, AV	-	> 0	Detected (+)
	-	N/A	Not detected (-)
IC	≤ 40	-	Detected (+)
	N/A	-	Not detected (-)

1) Ct: Cycle Threshold

2) Qt: Quantitative Threshold (Log10)

Result of target = 10^{QT} copies/rxn

Target Result		IC Result	Interpretation
Graph 1	Graph 2		
+	-	+	**Target Nucleic acid, Detected**
-	+		
+	+		
+	-	-	**Target Nucleic acid, Detected*** - Additional analyte(s) which are not detected may be present.
-	+		
+	+		
-	-	+	**Target Nucleic acid, Not detected**
-	-	-	**Invalid**** - Results suggest inadequate specimen collection, processes or presence of inhibitors. - Repeat the test from the nucleic acid extraction using another aliquot of the original specimen. - If the same result is shown in the diluted nucleic acid, please collect samples again.

* High level of target nucleic acids may cause interference in Internal Control detection and readout. Invalid IC signal do not indicate that the positive results for targets are invalid.

** See TROUBLESHOOTINGS (page 47) for the detailed instruction.

3.2. BV Interpretation

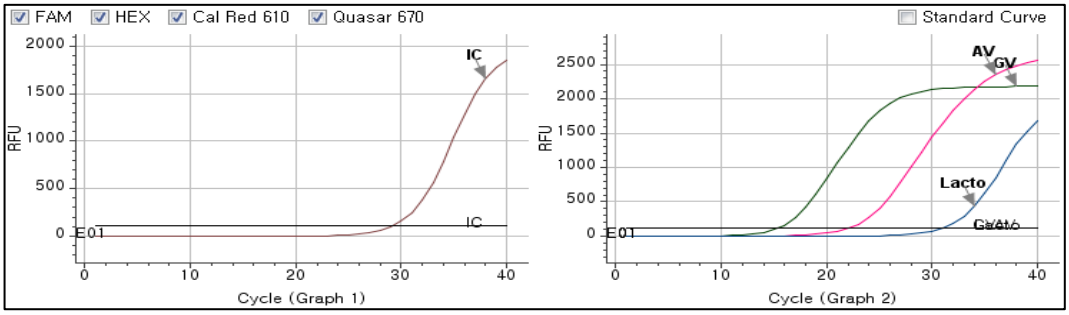
The Seegene Viewer software automatically interprets test results as Normal, Intermediate and Positive of BV status based on the amplification status of target(s).

Cut-off determination for Bacterial Vaginosis (BV) interpretation

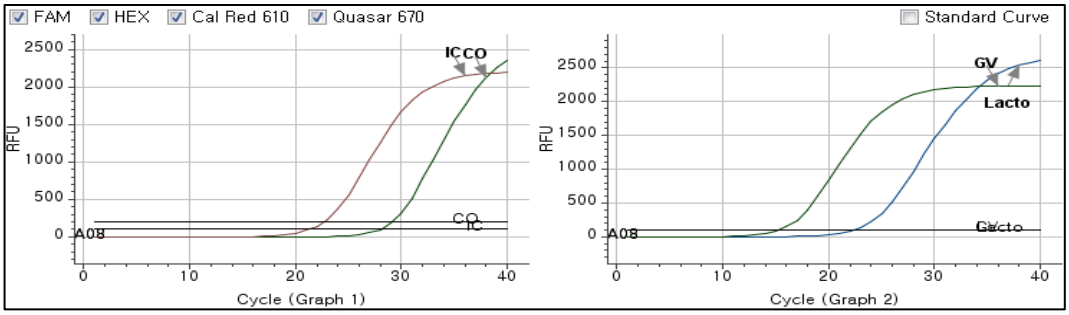
The results from the retrospective clinical study were used to validate the BV cut-offs for Allplex™ Vaginitis Screening Assay. For this validation, PCR metrics from vaginosis target analytes and results generated by the BV call algorithm were statistically analyzed in comparison to results from the applicable reference method. ROC curve analysis was performed to confirm the optimal cut-offs for each target analyte as well as for the cut-offs used to determine bacterial vaginosis status.

4. Application to Clinical Samples

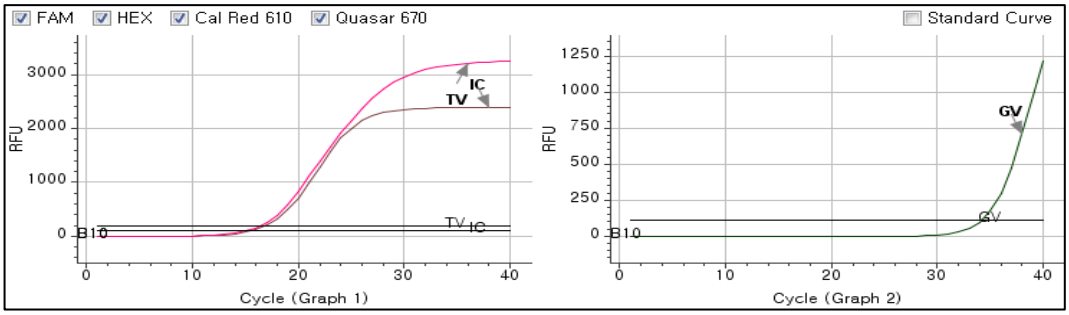
Sample 1



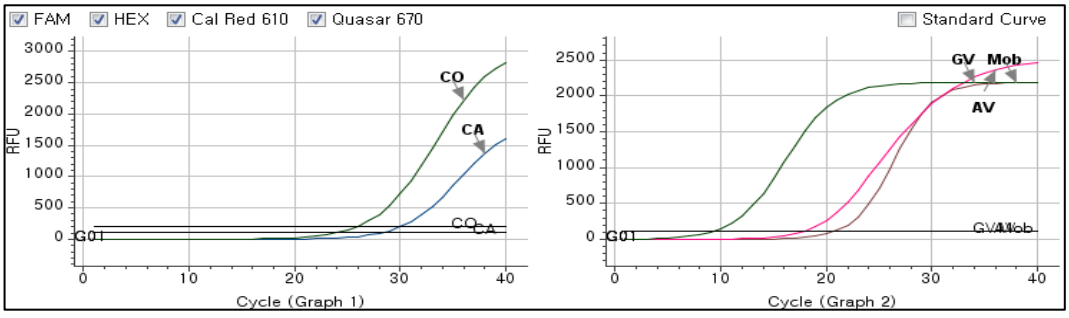
Sample 2



Sample 3



Sample 4



Sample	Seegene Viewer Result																				
	FAM				HEX				CAL Red 610				Quasar 670				Auto Interpretation	BV Interpretation	CA	CO	TV
	CA	Ct	Lacto	Qt	CO	Ct	GV	Qt	TV	Ct	AV	Qt	Mob	Ct	IC	Ct					
1	-	N/A	+	1.43	-	N/A	+	6.27	-	N/A	+	4.48	-	N/A	+	29.31	"Lacto, GV, AV"	Bacterial Vaginosis	-	-	-
2	-	N/A	+	3.94	+	29.20	+	6.22	-	N/A	-	N/A	-	N/A	+	21.50	"Lacto, CO, GV"	Bacterial Vaginosis	-	CO	-
3	-	N/A	-	N/A	-	N/A	+	0.73	+	16.50	-	N/A	-	N/A	+	16.57	"GV, TV"	Normal*	-	-	TV
4	+	28.42	-	N/A	+	25.88	+	8.07	-	N/A	+	5.63	+	20.81	-	N/A	"CA, CO, GV, AV, Mob"	Bacterial Vaginosis	CA	CO	-

*: The lack of *Lactobacillus* spp. may be due to the patient being postmenopausal.

TROUBLESHOOTINGS

Allplex™ Vaginitis Screening Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under correct settings.
	Incorrect storage or expiration of the test kit	Please check the storage conditions (See page 10) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Nucleic acid extraction failure	Make sure that you use a recommended extraction method.
	Error in specimen collection	If both target and IC signal were not observed that means specimen collected inappropriately. Recollect the specimen.
No Internal Control signal	Sampling error or high load of pathogen's nucleic acid	If both target pathogen signal and IC signal are not observed, then collect samples again. If target pathogen signal is observed but not IC, then IC amplification may have been inhibited by high titer of target pathogen. If you want to confirm IC signal, dilute the specimen (1/10~1/100) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the specimen (1/10~1/100) in saline buffer and repeat the test from extraction step.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Centrifuge the PCR tube before run.

Allplex™ Vaginitis Screening Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative false positive or target signals observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.
Putative false negative or no signal observed in Positive Control	Error in specimen collection	Please check the specimen collection method and re-collect the specimen.
	Incorrect storage of the specimen	Please re-collect the specimen and repeat the entire procedure. Ensure that the specimen is stored as recommended.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Presence of inhibitor	Please dilute the specimen (1/10~1/100) in saline buffer and repeat the test from extraction step.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture (Sensitivity is compromised with pre-composed premix). All reagents must be homogenized and spun down before use.
Putative False Standard DNA signals	Incorrect storage of the standard DNA	Please check the storage conditions (See page 10) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	$R^2 < 0.980$	Make sure that SD1, SD2 and SD3 are set correctly and correct if necessary. If it does not help, repeat PCR for all samples, SD1, SD2 and SD3.

PERFORMANCE

1. Specificity

Cross-reactivity of Allplex™ Vaginitis Screening Assay was tested using 167 standard materials and organisms as indicated below. Specific targets which were designed for the detection were identified by Allplex™ Vaginitis Screening Assay.

No.	Organism	Source	Isolated No.	Result†
1	*Atopobium vaginae*	KCTC	15240	AV Detected
2	*Candida albicans*	Geomebiotics	18G-7	CA Detected
3	*Candida dubliniensis*	KCTC	17427	CO Detected
4	*Candida glabrata*	KCCM	50044	CO Detected
5	*Candida krusei*	KCCM	50633	CO Detected
6	*Candida lusitaniae*	KCCM	50541	CO Detected
7	*Candida parapsilosis*	KCTC	7653	CO Detected
8	*Candida tropicalis*	KCCM	32008	CO Detected
9	*Gardnerella vaginalis*	KCTC	5096	GV Detected
10	*Lactobacillus crispatus*	KCTC	5054	Lacto Detected
11	*Lactobacillus gasseri*	KCTC	3163	Lacto Detected
12	*Lactobacillus jensenii*	KCTC	5194	Lacto Detected
13	*Mobilluncus curtisii*	ATCC	35241	Mob Detected
14	*Mobiluncus mulieris*	ATCC	35240D-5	Mob Detected
15	*Trichomonas vaginalis*	ATCC	30001	TV Detected
16	*Acinetobacter baumannii*	KCCM	35401	Not Detected
17	*Acinetobacter calcoceticus*	KCTC	2357	Not Detected
18	*Acinetobacter lwoffii*	KCTC	12407	Not Detected
19	*Acinetobacter schindleri*	KCTC	12409	Not Detected
20	*Acinetobacter ursingii*	KCTC	12410	Not Detected
21	*Actinomyces isrealii*	KCTC	9054	Not Detected
22	*Aerococcus viridans*	ATCC	11563	Not Detected
23	*Aeromonas hydrophilia*	KCTC	2358	Not Detected
24	*Agrobacterium tumefaciens*	KCCM	12137	Not Detected
25	*Alcaligenes faecalis*	KCTC	2678	Not Detected
26	*Atopobium minutum*	KCTC	5038	Not Detected

No.	Organism	Source	Isolated No.	Result†
27	*Atopobium parvulum*	KCTC	3663	Not Detected
28	*Bacillus subtilis*	KCTC	1021	Not Detected
29	*Bacteroides caccae*	ATCC	43185	Not Detected
30	*Bacteroides fragilis*	KCTC	5013	Not Detected
31	*Bacteroides ovatus*	KCTC	5827	Not Detected
32	*Bacteroides vulgatus*	ATCC	8480	Not Detected
33	*Bacteroides xylanisolvens*	KCTC	15192	Not Detected
34	*Bifidobacterium adolescentis*	KCCM	11206	Not Detected
35	*Bifidobacterium longum*	KCCM	11953	Not Detected
36	*Bifidobacterium minimum*	KCTC	3273	Not Detected
37	*Blautia producta(prevot)* Liu et al.	ATCC	27340	Not Detected
38	*Brevibacterium linens*	KCTC	9099	Not Detected
39	*Candida orthopsilosis*	ATCC	96139	Not Detected
40	*Chlamydia trachomatis* (LGV I)	ATCC	VR-901BD	Not Detected
41	*Chlamydia trachomatis* (LGV II)	ATCC	VR-902BD	Not Detected
42	*Chlamydia trachomatis* (LGV III)	ATCC	VR-903D	Not Detected
43	*Chlamydia trachomatis* (serovar A)	ATCC	VR-571B	Not Detected
44	*Chlamydia trachomatis* (serovar B)	ATCC	VR-573	Not Detected
45	*Chlamydia trachomatis* (serovar Ba)	ATCC	VR-347	Not Detected
46	*Chlamydia trachomatis* (serovar C)	ATCC	VR-1500	Not Detected
47	*Chlamydia trachomatis* (serovar D)	ATCC	VR-885	Not Detected
48	*Chlamydia trachomatis* (serovar E)	ATCC	VR-348B	Not Detected
49	*Chlamydia trachomatis* (serovar F)	ATCC	VR-346	Not Detected
50	*Chlamydia trachomatis* (serovar G)	ATCC	VR-878	Not Detected
51	*Chlamydia trachomatis* (serovar H)	ATCC	VR-879D	Not Detected
52	*Chlamydia trachomatis* (serovar I)	ATCC	VR-880	Not Detected
53	*Chlamydia trachomatis* (serovar J)	ATCC	VR-886	Not Detected
54	*Chlamydia trachomatis* (serovar K)	ATCC	VR-887	Not Detected
55	*Chlamydophila pneumoniae*	ATCC	VR-1360	Not Detected
56	*Chromobacterium violaceum*	KCTC	2897	Not Detected
57	*Citrobacter freundii*	KCTC	2509	Not Detected
58	*Clostridium difficile* (Toxin A+ / B+)	ATCC	9689D-5	Not Detected
59	*Clostridium perfringens*	KCTC	3269	Not Detected

No.	Organism	Source	Isolated No.	Result†
60	*Corynebacterium genitalium*	ATCC	33031	Not Detected
61	*Corynebacterium xerosis*	KCTC	3435	Not Detected
62	*Cryptococcus neoformans*	KCCM	50564	Not Detected
63	*Cytomegalovirus*	ATCC	VR-807	Not Detected
64	*Deinococcus radiopugnans*	ATCC	19172	Not Detected
65	*Derxia gummosa*	KCTC	12784	Not Detected
66	*Enterobacter aerogenes*	KCTC	2190	Not Detected
67	*Enterobacter cloacae*	KCCM	12178	Not Detected
68	*Enterococcus avium*	ATCC	14025	Not Detected
69	*Enterococcus faecalis*	ATCC	11700	Not Detected
70	*Enterococcus faecium*	ATCC	5155951559	Not Detected
71	Epstein Barr Virus	ATCC	VR-1492	Not Detected
72	*Escherichia coli*	KCCM	11591	Not Detected
73	*Gemella haemolysans*	ATCC	10379	Not Detected
74	*Haemophilus ducreyi*	KCTC	2745	Not Detected
75	*Haemophilus influenzae*	ATCC	51907D	Not Detected
76	Hepatitis A virus	ATCC	VR-1541	Not Detected
77	Hepatitis B virus	ATCC	VR-3232SD	Not Detected
78	Human Herpesvirus 1	KBPV	VR-52	Not Detected
79	Human Herpesvirus 2	KBPV	VR-53	Not Detected
80	Varicella-zoster virus	ATCC	VR-1367	Not Detected
81	Human papillomavirus strain 18	KCLB	10002	Not Detected
82	*Kingella denitrificans*	ATCC	33394	Not Detected
83	*Kingella kingae*	ATCC	23330	Not Detected
84	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	ATCC	6908	Not Detected
85	*Lactobacillus acidophilus*	KCCM	32820	Not Detected
86	*Lactobacillus amylovorus*	KCCM	40431	Not Detected
87	*Lactobacillus brevis*	KCTC	3498	Not Detected
88	*Lactobacillus casei*	KCCM	12452	Not Detected
89	*Lactobacillus delbrueckii* subsp. *lactis*	KCTC	3035	Not Detected
90	*Lactobacillus delbrueckii* subsp. *delbrueckii*	KCTC	13730	Not Detected

No.	Organism	Source	Isolated No.	Result†
91	*Lactobacillus fermentum*	KCCM	40401	Not Detected
92	*Lactobacillus gallinarum*	KCCM	40987	Not Detected
93	*Lactobacillus helveticus*	KCTC	15060	Not Detected
94	*Lactobacillus hominis* DSM 23910	KCTC	21045	Not Detected
95	*Lactobacillus iners*	CCARM	123	Not Detected
96	*Lactobacillus intestinalis*	KCCM	40990	Not Detected
97	*Lactobacillus johnsonii*	KCCM	32825	Not Detected
98	*Lactobacillus kalixensis*	KCTC	5856	Not Detected
99	*Lactobacillus oris*	KCCM	40993	Not Detected
100	*Lactobacillus parabuchneri*	KCTC	3503	Not Detected
101	*Lactobacillus pentosus*	KCCM	40997	Not Detected
102	*Lactobacillus plantarum*	ATCC	700934	Not Detected
103	*Lactobacillus reuteri*	KCCM	40717	Not Detected
104	*Lactobacillus rhamnosus*	KCCM	32405	Not Detected
105	*Lactobacillus salivarius* subsp. *salicinius*	KCCM	40998	Not Detected
106	*Lactobacillus sanfrancisensis*	ATCC	27651	Not Detected
107	*Lactobacillus ultunensis*	KCTC	5857	Not Detected
108	*Lactobacillus vaginalis*	KCTC	41001	Not Detected
109	*Legionella pneumophila*	KCCM	41781	Not Detected
110	*Micrococcus luteus*	KCTC	3063	Not Detected
111	*Moraxella catarrhalis*	KCCM	42707	Not Detected
112	*Moraxella osloensis*	ATCC	19976	Not Detected
113	*Morganella morganii* subsp. *morganii*	KCCM	11497	Not Detected
114	*Mycobacterium smegmatis*	KCTC	9108	Not Detected
115	*Mycoplasma arginini*	ATCC	23838D	Not Detected
116	*Mycoplasma felis* Cole et al.	ATCC	23391	Not Detected
117	*Mycoplasma genitalium*	ATCC	33530	Not Detected
118	*Mycoplasma hominis*	ATCC	27545-TTR	Not Detected
119	*Mycoplasma iowae* Jordan et al.	ATCC	33552	Not Detected
120	*Mycoplasma leonicaptivi* Hill	ATCC	49890	Not Detected
121	*Mycoplasma pneumoniae*	ATCC	15531	Not Detected

NO.	Organism	Source	Isolate No.	Result†
122	*Mycoplasma pulmonis*	ATCC	19612	Not Detected
123	*Mycoplasma spumans*	ATCC	19526	Not Detected
124	*Neisseria cinerea*	ATCC	14685	Not Detected
125	*Neisseria elongata*	KCTC	23361	Not Detected
126	*Neisseria flavescens*	ATCC	13120	Not Detected
127	*Neisseria gonorrhoeae*	ATCC	700825	Not Detected
128	*Neisseria lactamica*	ATCC	23970	Not Detected
129	*Neisseria meningitidis*	KCCM	41562	Not Detected
130	*Neisseria mucosa*	ATCC	19696	Not Detected
131	*Neisseria perflava*	ATCC	10555	Not Detected
132	*Neisseria polysaccharea*	ATCC	43768	Not Detected
133	*Neisseria sicca*	ATCC	29256	Not Detected
134	*Neisseria subflava*	ATCC	19243	Not Detected
135	*Paracoccus denitrificans*	KCTC	2530	Not Detected
136	*Peptostreptococcus anaerobius*	ATCC	49031D-5	Not Detected
137	*Prevotella bivia*	KCTC	5454	Not Detected
138	*Prevotella buccalis*	KCTC	5496	Not Detected
139	*Prevotella disiens*	KCTC	5499	Not Detected
140	*Prevotella intermedia*	KCTC	5692	Not Detected
141	*Prevotella melaninogenica*	KCTC	5457	Not Detected
142	*Propionibacterium acnes*	KCTC	3314	Not Detected
143	*Proteus mirabilis*	ATCC	12453	Not Detected
144	*Providencia stuartii*	KCTC	2568	Not Detected
145	*Pseudomonas aeruginosa*	KCCM	11328	Not Detected
146	*Pseudomonas putida*	KCTC	1643	Not Detected
147	*Rahnella aquatilis*	KCTC	2863	Not Detected
148	*Rhodospirillum rubrum*	KCTC	1372	Not Detected
149	*Saccharomyces cerevisiae*	KCCM	50511	Not Detected
150	*Salmonella enteritidis*	KCCM	12021	Not Detected
151	*Salmonella typhimurium*	KCCM	40253	Not Detected
152	*Staphylococcus aureus*	KCTC	1621	Not Detected
153	*Staphylococcus epidermidis*	ATCC	12228	Not Detected
154	*Streptococcus agalactiae*	KCCM	40417	Not Detected

NO.	Organism	Source	Isolate No.	Result†
155	*Streptococcus bovis*	KCCM	40409	Not Detected
156	*Streptococcus mitis*	KCTC	5650	Not Detected
157	*Streptococcus mutans*	KCTC	3065	Not Detected
158	*Streptococcus pneumoniae*	ATCC	BAA-255D	Not Detected
159	*Streptococcus pyogenes*	ATCC	19615	Not Detected
160	*Streptococcus salivarius*	KCCM	11926	Not Detected
161	*Streptococcus sanguinis*	KCTC	3299	Not Detected
162	*Treponema pallidium*	ATCC	BAA-2642SD	Not Detected
163	*Trichomonas tenax*	ATCC	30207	Not Detected
164	*Ureaplasma parvum*	ATCC	27818	Not Detected
165	*Ureaplasma urealyticum*	ATCC	27816	Not Detected
166	*Vibrio parahaemolyticus*	KCTC	2471	Not Detected
167	*Yersinia enterocolitica*	ATCC	23715	Not Detected

† Specificity tests were repeated 3 times.

※ ATCC: American Type Culture Collection
ZMC : ZeptoMetrix Corporation
KCTC : Korean Collection for Type Culture
KCCM : Korean Culture Center of Microorganisms
KCLB: Korean Cell Line Bank

2. Sensitivity

The sensitivity is defined as the lowest concentration of organism that can be consistently detected (≥ 95% of positive results among all tested sample). It was confirmed when the correct organism/assay results were obtained from at least 30 of the 30 samples (30/30 = 100%) tested. The sensitivity of Allplex™ Vaginitis Screening Assay was determined using target plasmid DNAs. Detection limit for Allplex™ Vaginitis Screening Assay was 100 copies/reaction.

3. Reproducibility

The reproducibility panel of 21 simulated analytes was prepared that included High negative (0.1 X LoD), Low positive (1X LoD) and Moderate positive (3X LoD) samples. At each testing site, the panel was tested for five days, two runs per day by two different operators and triplicate of each panel per run. It was tested with a single lot of Allplex™ Vaginitis Screening Assay at three different sites and three lots at one in-house site. The positive rates were observed for each analyte for reproducibility study: 100.00% for Moderate positive samples, ≥100.00% for Low positive samples and ≥79.33% for High negative samples.

The reproducibility of the Allplex™ Vaginitis Screening Assay was evaluated among sites, product lots and experimenters. The results were satisfied with the criteria set above, thus confirming the reproducible performances of Allplex™ Vaginitis Screening Assay.

4. Interfering substances

This test was conducted using interfering substances composed of 4 substances in order to confirm the performance of the Allplex™ Vaginitis Screening Assay in the presence of potential interfering substances. There was no effect on the result by adding the substances: non-specific detection or inhibition on target amplification. Based on the results, 4 interfering substances had no effect on Allplex™ Vaginitis Screening Assay results.

No.	Interfering substances	Concentration
1	Human whole blood	2.5 % (v/v)
2	Albumin (BSA)	60 g/L
3	Bilirubin	342 µmol/L
4	Mucus	-

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KEY TO SYMBOLS

Key to symbols used in the manual and labels.

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use-by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
PREMIX	PCR Master Mix or Detection Mix
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
SD	Standard DNA
	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
UDI	Unique Device Identifier
rxns	Reaction barcode for automated extraction system

ORDERING INFORMATION

Cat. No.	Product	Size
Allplex™ series		
SD10321Z	**Allplex™ Vaginitis Screening Assay**	**25 rxns**
SD9750Y	Allplex™ Vaginitis Screening Assay	50 rxns
SD9750X	**Allplex™ Vaginitis Screening Assay**	**100 rxns**
Accessory product		
SG1701	Ribo_spin vRD (Viral RNA/DNA Extraction Kit)	50 preps
Automated extraction Systems		
65415-02	Microlab NIMBUS IVD	EA
173000-075	Microlab STARlet IVD	EA
65415-03	Seegene NIMBUS	EA
67930-03	Seegene STARlet	EA
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384T / 1box
SG72100	AIOS	EA