For Professional Use Only

Allplex™

HPV HR Detection

(Cat. No. HP10370X/HP10376L, HP10371Z)

Allplex™ PCR System for detection of human papillomavirus - 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) from cervical specimens and self-collected vaginal specimens.

For use with

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

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

CE

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

REF **HP10370X** Σ 100 REF **HP10371Z** Σ 25

REF **HP10376L** Σ 100 x 8 kits

Seegene Inc.

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Not available in the U.S.

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NOTICES

- For *in vitro* diagnostic use only.
- Allplex™ HPV HR Detection should be performed by qualified, trained personnel.
- If this product is used with **Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, Seegene STARlet and Seegene STARlet 96MPH**, it provides a maximum of 5 separate runs.
- **This test has been validated for the following specimen types: cervical specimens and self-collected vaginal specimens**. This test has not been validated for any other types of specimens.
- **Store DNA samples at -20℃ until use and keep 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.
- Reliability of the results depends on adequate specimen collection, transport, storage and processing procedure.
- 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 protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. The use of sterile disposable pipette tips is recommended.
- Do not pool reagents from different lots.
- Do not use the product after its expiration date.
- Do not reuse all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparations.
- To prevent contamination of reagents, the use of filter-tips is recommended. Also, be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive controls.
- Use separated 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 unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Expiration date is 13 months at ≤ -20℃ from the date of manufacture. Please refer to the final label for expiration date.
- Seegene NIMBUS and Seegene STARlet are the same equipment as the Microlab NIMBUS IVD and Microlab STARlet IVD, just the manufacturer is different. Since there are no hardware changes on the instrument, 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 from two softwares are the same.
- This kit is intended to aid in the differential diagnosis of target pathogen infections; Human papillomaviruses.
- Self-collection should be completed in a health care setting with instruction of healthcare provider.
- **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.

INTENDED USE

Allplex™ HPV HR Detection is a qualitative *in vitro* molecular diagnostic assay designed to detect human papillomaviruses in cervical specimens or self-collected vaginal specimens. Allplex™ HPV HR Detection detects HPV16, HPV18, and other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

Allplex™ HPV HR Detection is indicated:

a) To be used with cervical cytology to adjunctively screen to assess the presence or absence of HPV16, HPV18 and other 12 individual high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

b) To be used as a primary screening test to identify women at increased risk for the development of cervical cancer or the presence of high-grade disease.

c) To be used as a primary screening test to assess the presence or absence of HPV16, HPV18 and other 12 individual high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The results from Allplex™ HPV HR Detection, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

PRINCIPLES AND PROCEDURE OVERVIEW

1. Principles

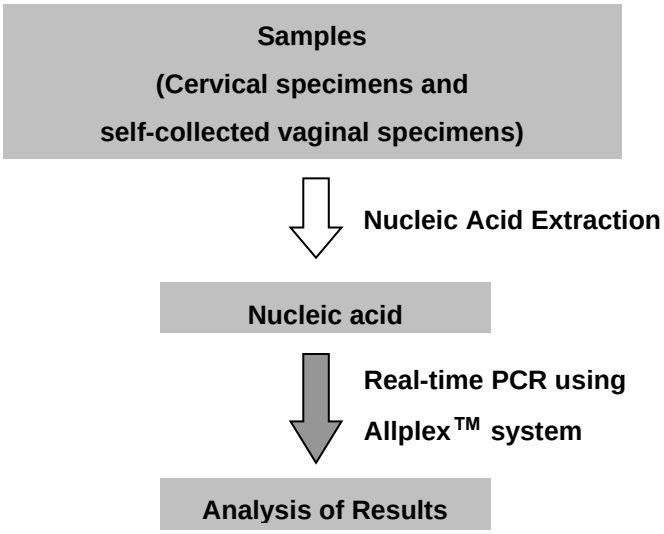
Allplex™ HPV HR Detection is a multiplex real-time PCR assay that enables simultaneous amplification and detection of target nucleic acids of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) as well as Internal Control (IC).

To perform the multiplex target amplification and detection in a single reaction, this assay kit employs Seegene's innovative proprietary DPO™, TOCE™, MuDT™ and 3 Ct technologies. 3 Ct technology can provide the Ct value of three targets in one channel without affecting sensitivity and specificity. The presence of specific gene sequences in the reaction is reported as a Ct value through Seegene Viewer analysis software.

In PCR, amplification efficiency can be reduced by inhibitors that may be present in the clinical specimens. An Internal Control (IC) is incorporated into the product as an endogenous whole process control in order to monitor nucleic acid isolation, and to check for possible PCR inhibition. The IC is co-amplified with the target nucleic acids within the clinical specimens. Allplex™ HPV HR Detection uses human house-keeping gene as an endogenous IC which can ensure extraction of DNA, verification of PCR reaction and clarification of cell adequacy from each specimen.

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

2. Procedure Overview



BACKGROUND INFORMATION

Human Papilloma Virus (HPV) infection is linked with cervical cancer. HPV can be divided into “high-risk (HR)” and “low-risk (LR)” groups on the basis of their association with cervical lesions. Therefore, it is very important to know which type of HPV is infected in patients to prevent cancer development and transmission of disease. Currently, commercially available major products to diagnose HPV are based on probe-hybridization method to detect and/or genotype HPV. However, main defects of the probe-hybridization based methods are high false positive rate due to cross-reactivity between probes and various kinds of viral DNA or PCR amplicons used for hybridization. Here we are introducing an innovative HPV detection/genotyping assay system which amplifies only specific targets without any cross reactivity and is automated in detection using real-time PCR method. The product only specifically detects true HPV and accurately genotypes them. It also contains endogenous Internal Control (IC) to check any inhibition that might occur during PCR reaction.

Cervical cancer, which progresses from the precancerous stage to invasive cancer, has 7-20 years of precancerous stage; Consequently early diagnosis is possible when HPV infection is suspected. High-risk HPV group may lead to the development of cervical cancer; especially, HPV16 and 18 are associated with 70% of cervical cancer case. On the other hands, low-risk HPV group including HPV6 and 11 may cause genital warts. Allplex™ HPV HR Detection can identify 14 high-risk HPV types including HPV16 and 18 at the same time.

REAGENTS

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

Order information (REF HP10370X/HP10376L*).

* HP10376L is a package containing 8 kits of HP10370X (100 reactions).

Allplex™ HPV HR Detection			
Symbols	**Contents**	**Volume**	**Description**
PRIMER	HPV HR MOM	500 μL	Oligo Mix: - Amplification and detection reagents
ENZYME	EM4	500 μL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
BUFFER	EM4 Buffer	500 μL	Buffer for Real-time PCR - Buffer containing BSA and Glycerol
CONTROL +	Allplex HPV HR PC1	50 μL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC2	50 μL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC3	50 μL	Positive Control (PC): - Mixture of pathogen clones
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 Inc. or regional manager. Please use Seegene Viewer beyond V3.

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

Order information (REF HP10371Z).

Allplex™ HPV HR Detection			
Symbols	**Contents**	**Volume**	**Description**
PRIMER	HPV HR MOM	125 μL	Oligo Mix: - Amplification and detection reagents
ENZYME	EM4	125 μL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
BUFFER	EM4 Buffer	125 μL	Buffer for Real-time PCR - Buffer containing BSA and Glycerol
CONTROL +	Allplex HPV HR PC1	50 μL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC2	50 μL	Positive Control (PC): - Mixture of pathogen clones
CONTROL +	Allplex HPV HR PC3	50 μL	Positive Control (PC): - Mixture of pathogen clones
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 Inc. or regional manager. Please use Seegene Viewer beyond V3.

STORAGE AND HANDLING

All components of Allplex™ HPV HR Detection should be stored at ≤-20℃. All components are stable under recommended storage conditions until the expiration date stated on the label. The performance of kit components is not affected for up to 5 times of freezing and thawing. If the reagents are to be used only intermittently, they should be frozen in aliquots.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tube
- Nucleic acid extraction kit (see Nucleic Acid Extraction)
- Ice maker
- Desktop centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection system (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, 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)
- Vial Cap Management System (Cat. No. 6600532-01, Hamilton)
- 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)*
- 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 have to be treated as potentially infectious materials. Only those sample materials are permitted, which are collected, stored and transported attending strictly the following rules and instructions:

Cervical specimen
Self-collected vaginal specimen

Note: To ensure a high sample quality, the specimens should be transported as fast as possible. The specimens have to be transported at the indicated temperature conditions.

A. Specimen Collection

Cervical specimen

For the collection of cervical specimen, please use following materials:

- Cervical specimen can be collected and transported in the following mediums:
  - eNAT™ (COPAN, Italia), ThinPrep® (HOLOGIC, USA), SurePath™ (Becton-Dickinson, USA) or CellPreserv (Kolplast, Brazil) media

Cervical specimen collection kit	Manufacturer	Cat. No.
eNAT PM 2ML L-SHAPE APPLICATOR	COPAN	606CS01L

- Leave the swab in the transport medium. Close and label the sample container. Stick closely to the instructions given for storage and transport.
- Please follow a recommended protocol to collect columnar and squamous epithelium cells after removal of the cervical mucus.

Self-collected vaginal specimen

- For the collection of self-collected vaginal specimen, please use following material:
  - Rovers® Evalyn® Brush (Rovers Medical Devices B.V., Netherlands)

Self-sampling device	Manufacturer	Cat. No.
Rovers® Evalyn® Brush	Rovers Medical Devices B.V.	380500131

- Self-collected vaginal specimen can be collected and stored in ThinPrep® PreservCyt® Solution.
- Follow each manufacturer's instructions of sampling device and transport media for collection and storage of vaginal cell specimens.

B. Specimen Storage & Transport

Specimen	Media	Storage & Transport duration*	
		2~8℃**	Room temperature**
Cervical specimen	eNAT™	90 days	90 days
	ThinPrep®	90 days	90 days
	SurePath™	90 days	90 days
	CellPreserv	90 days	90 days
Self-collected vaginal specimen	ThinPrep®	90 days	90 days

* Duration: Specimen collected from the period prior to the test including specimen storage and transport prior to the test.

** Optimum temperature for transport is 2~25℃.

Note: Performance may be affected by prolonged storage of specimens.

Note: Specimens should also adhere to local and national instructions for transport of pathogenic material.

2. Nucleic Acid Extraction

Various manufacturers offer nucleic acid extraction kits. Use right amount of sample according to the protocol in use. The following extraction kits have been validated for use with this kit.

[Extraction methods in different medium]

Note: Please use the automated extraction system according to the medium shown in the following table.

		Automated Extraction System			
Specimen	**Transport media**	**Microlab NIMBUS IVD / STARlet IVD**	**Seegene NIMBUS / STARlet**	**SEEPREP32**	**STARlet 96MPH**
		Universal Cartridge Kit	Universal Cartridge Kit	STARMag 96 ProPrep	STARMag™ S96H N Kit*
Cervical specimen	eNAT	O	O	O	O
	ThinPrep®	O	O	O	O
	SurePath™	O	O	X	O
	CellPreserv	O	O	O	O
Self-collected vaginal specimen	ThinPrep®	O	O	O	O

Optional: Vial Cap Management System can be used with Microlab STARlet IVD, and Seegene STARlet.

Optional: AIOS can be used with Seegene STARlet.

*STARMag™ S96H N Kit is designed and validated for the use with the configuration of Seegene STARlet with CO-RE 96 Probe Head and Seegene STARlet 96MPH.

A. Pre-treatment of ThinPrep® and SurePath™

- Equilibrate samples to room temperature (19~25℃).
- Centrifuge 1 mL of specimen for 15 minutes at 15,000 x *g* (13,000 rpm).
- The supernatant has to be discarded. Afterwards, the recommend volume (200~300 µL, See Recommended Vol. of 2-C) should be resuspended in lysis buffer or 1X PBS by vortexing thoroughly to redissolve.

Note: Process pre-treatment step using 1X PBS if the samples are collected in ThinPrep® medium.

Note: Process pre-treatment step using lysis buffer from extraction kit if the samples are collected in SurePath™ medium.

Note: ThinPrep® and SurePath™ media can be processed without pre-treatment when using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, Seegene STARlet or Seegene STARlet 96MPH.

Note: CellPreserv and eNAT does not require a pre-treatment step.

B. Specimen Preparation

- Equilibrate samples to room temperature (19~25℃).
- For Cervical specimens and self-collected vaginal specimen which contain a swab/brush in the transport media, specimens should be mixed by vortexing.
- The caps from specimen tubes have to be removed carefully to avoid contamination. Any excess mucus in the specimen should be removed at this time by collecting it on the swab/brush. Any residual liquid from the mucus and the swab/brush should then be expressed by pressing the swab/brush against the side of the tube. Finally, the swab/brush and the mucus should be removed and discarded.
- Specimens from eNAT solution may be processed directly out of their primary container.

C. Automated Nucleic Acid Extraction System

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

C-1. 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

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

C-2. Microlab STARlet IVD

Option: Pre-analytic System (See Vial Cap Management System operation manual)

Automated Pre-analytic System	Manufacturer	Cat. No.	Recommended Vol.
Vial Cap Management System	Hamilton	6600532-01*	-

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

NOTE: Vial Cap Management System can be used with ThinPrep® and CellPreserv.

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

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

C-3. 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-4. Seegene STARlet

Option: Pre-analytic System (See Vial Cap Management System operation manual)

Automated Pre-analytic System	Manufacturer	Cat. No.	Recommended Vol.
Vial Cap Management System	Hamilton	6600532-01*	-

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

NOTE: Vial Cap Management System can be used with ThinPrep® and CellPreserv.

Option: Automated Linkage Structure (See AIOS operation manual)

Automated Linkage Structure	Manufacturer	Cat. No.
AIOS	Seegene	SG72100*

* If you would like to purchase the above products from Seegene Inc., please use this catalog number.

NOTE: Replace the cap of the Positive Control (PC) with a pierceable cap. After finishing the operation, replace the cap of the Positive Control (PC) with the original cap.

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

NOTE: If this product is used with AIOS applied Seegene STARlet, it provides a maximum of 3 separate runs.

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
STARMag™ S96H N Kit*	Seegene	EX00036P EX00037P	Specimen: 300 µL Elution: 100 µL

* STARMag™ S96H N Kit is designed and validated for the use with the configuration of Seegene STARlet with CO-RE 96 Probe Head and Seegene STARlet 96MPH.

C-5. SEEPREP32

Note: Proceed the extraction process using **'Pro-Protocol A'**.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
SEEPREP32	Seegene	SG71100	-
STARMag 96 ProPrep (Plate Type)	Seegene	EX00009P	Specimen: 200 µL Elution: 100 µL
STARMag 96 ProPrep (Tube Type)	Seegene	EX00009T	Specimen: 200 µL Elution: 100 µL

C-6. Seegene STARlet 96MPH

Note: See **Seegene STARlet 96MPH** operation manual.

Note: Seegene STARlet 96MPH is only applicable to HP10370X.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene STARlet 96MPH	Seegene	SG71101	-
STARMag™ S96H N Kit	Seegene	EX00036P EX00037P	Specimen: 300 µL Elution: 100 µL

3. Preparation for Real-time PCR

Note: When using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, Seegene STARlet and Seegene STARlet 96MPH for this step, refer to each operation manual.
Note: The 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 specimens. Use an extreme care to ensure no cross-contamination.
Note: Completely thaw the reagents on ice.
Note: Briefly spin down the reagent tubes to remove drops from the inner cap.
Note: **The steps A~D are automatically processed on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, Seegene STARlet, and Seegene STARlet 96MPH. Refer to each operation manual.**

A. Prepare PCR Mastermix.

5 μL	HPV HR MOM
5 μL	EM4
5 μL	EM4 Buffer
15 μL	Total volume of PCR Mastermix

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

B. Mix by inverting 5 times or quick vortex, and briefly spin down the tubes.

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

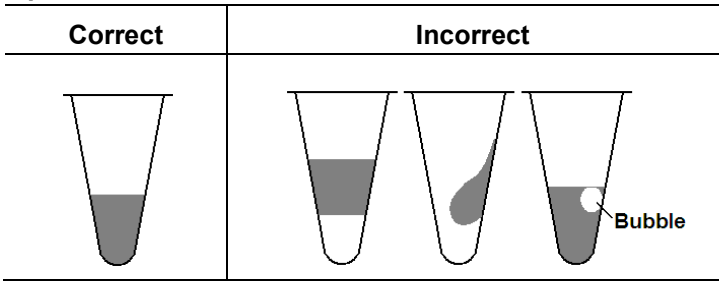
D. Add 5 μL of each sample's nucleic acids into the tube containing PCR Mastermix.

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

E. Close and briefly spin down the PCR tubes.

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

Note: It is recommended to spin down 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 "**Allplex HPV HR PC1", "Allplex HPV HR PC2" and "Allplex HPV HR PC3"** instead of sample's nucleic acid.

Note: Be careful not to cross-contaminate the Reaction Mastermix and samples with the Positive Control.

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

- **Positive Control**

There are 3 Positive Control tubes included in the kit; Allplex HPV HR PC1, PC2 and PC3.

Each PC includes clones for 5 targets (14 types of high risk and IC).

Note: To run the Positive Control reaction, prepare 3 PCR tubes. (See the results below.)

Positive control

Name	FAM			HEX			CalRed 610			Quasar 670			Quasar 705			Auto interpretation
	66	45	58	51	59	16	33	39	52	IC	35	18	56	68	31	
PC1	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	Positive Control (+)
PC2	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	Positive Control (+)
PC3	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	Positive Control (+)

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 3 steps: Protocol Setup, Plate Setup and Start Run.

A. Protocol Setup

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

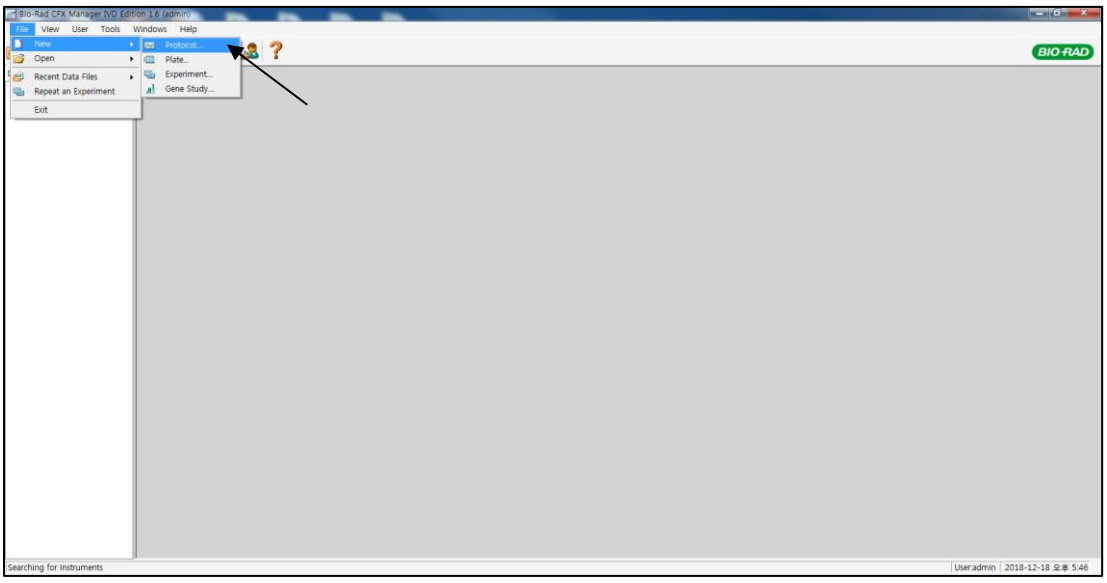


Fig. 1. **Protocol Setup**

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

Step	No. of cycles	Temperature	Duration
1	1	95°C	15 min
2	45	95°C	3 sec
3*		60°C	10 sec
4*		72°C	10 sec
5*		83°C	5 sec

Note*: Plate Read at Step 3, 4 and 5. Fluorescence is detected at 60°C, 72°C and 83°C.

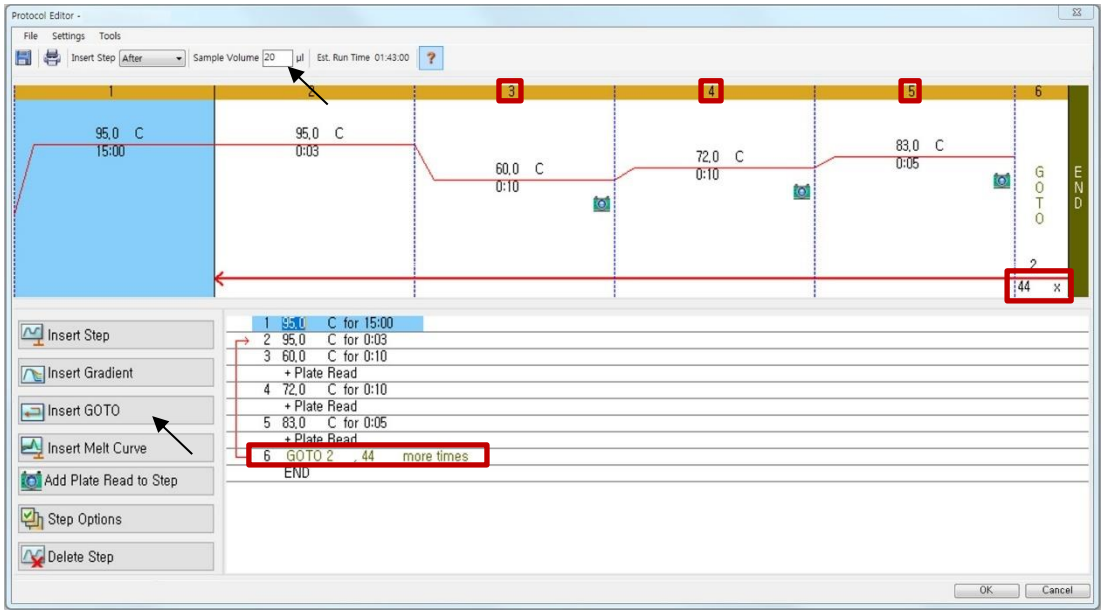


Fig. 2. **Protocol Editor**

Note: Click the "**Insert GOTO**" and type in "**GOTO 2, 44 more times**" at Step 6.

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.

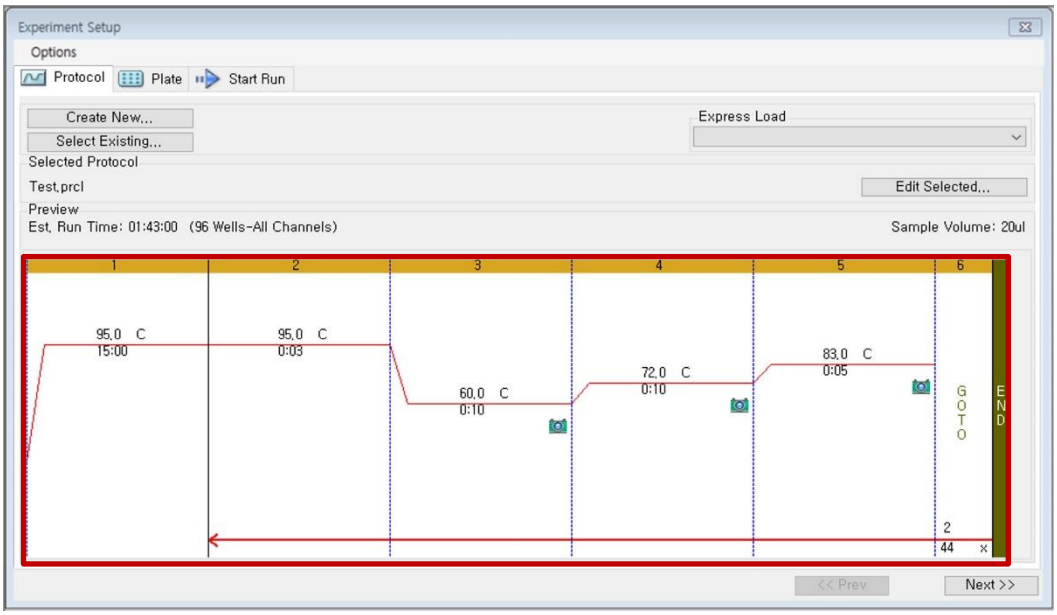


Fig. 3. **Experiment Setup: Protocol**

B. Plate Setup

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

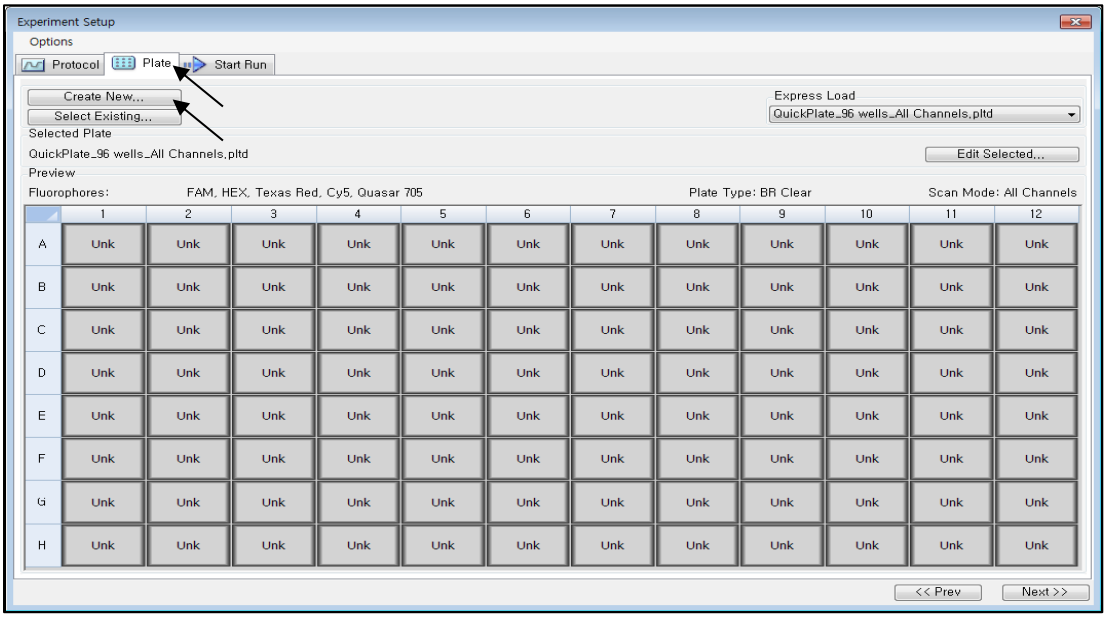


Fig. 4. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***) that will be used and click **"OK"**.

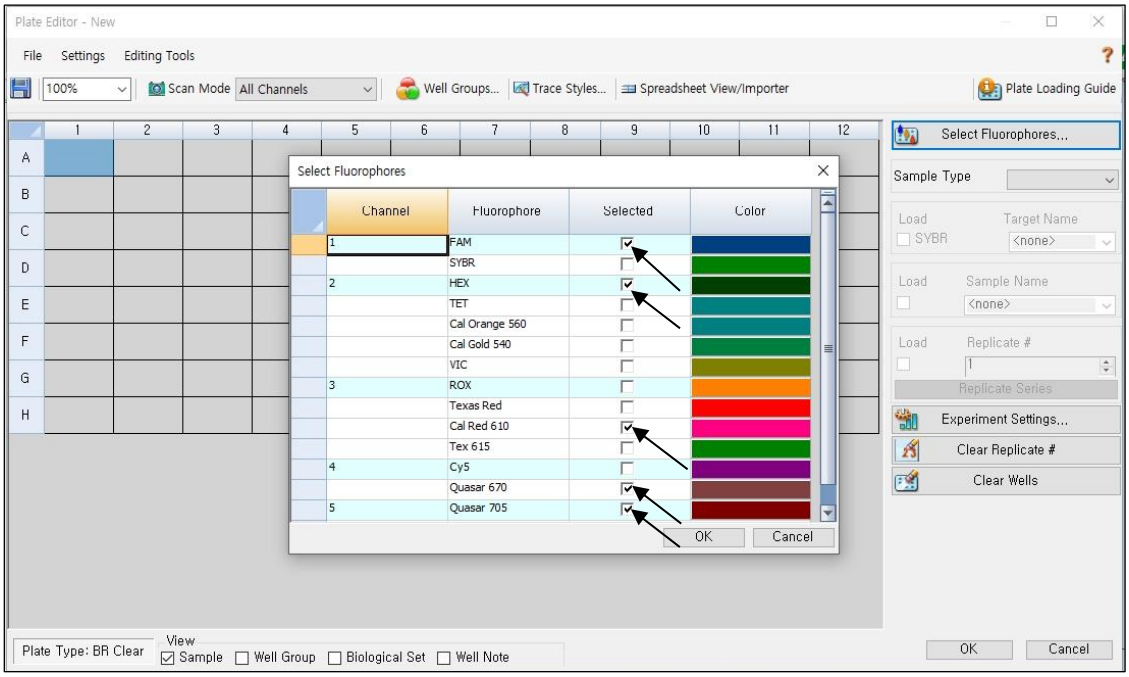


Fig. 5. **Select Fluorophores** (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***)

3) 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***

4) Click on the appropriate checkboxes (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***) to specify the fluorophores to be detected in the selected wells.

5) Type in "**Sample Name"** and **PC (PC1, PC2** and **PC3)**, and then press enter key.

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

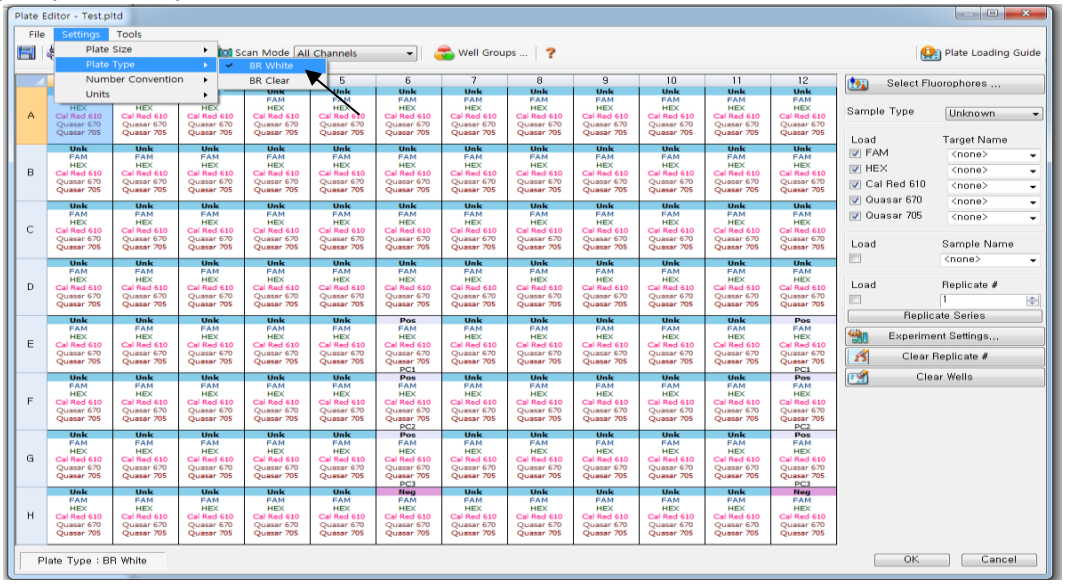


Fig. 6. **Plate Setup**

7) Click **"OK"** to save the new plate.

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

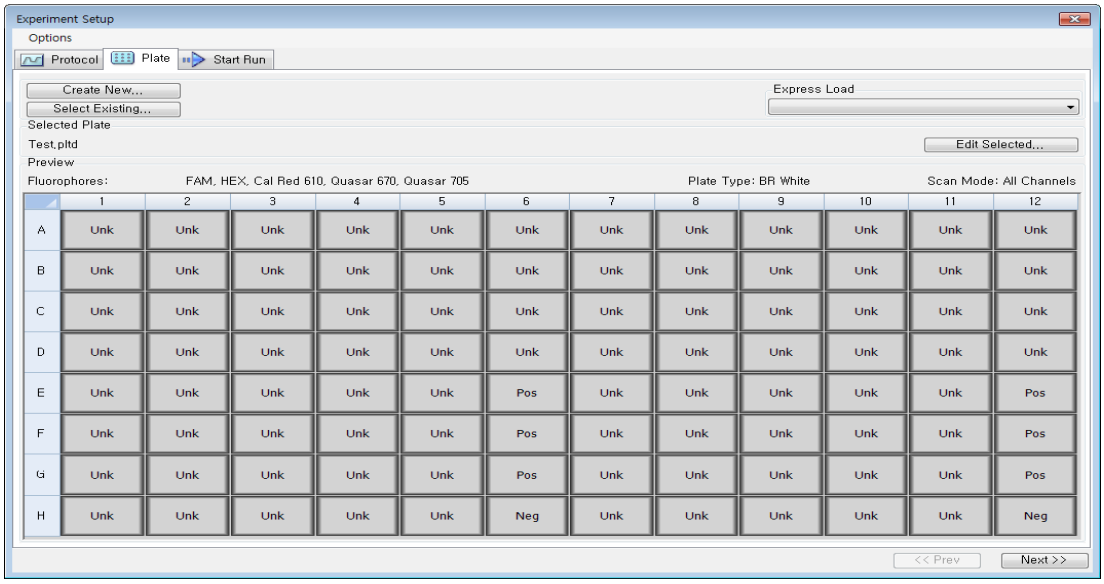


Fig. 7. **Experiment Setup: Plate**

9) Click **"Next"** to start run.

C. Start Run

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

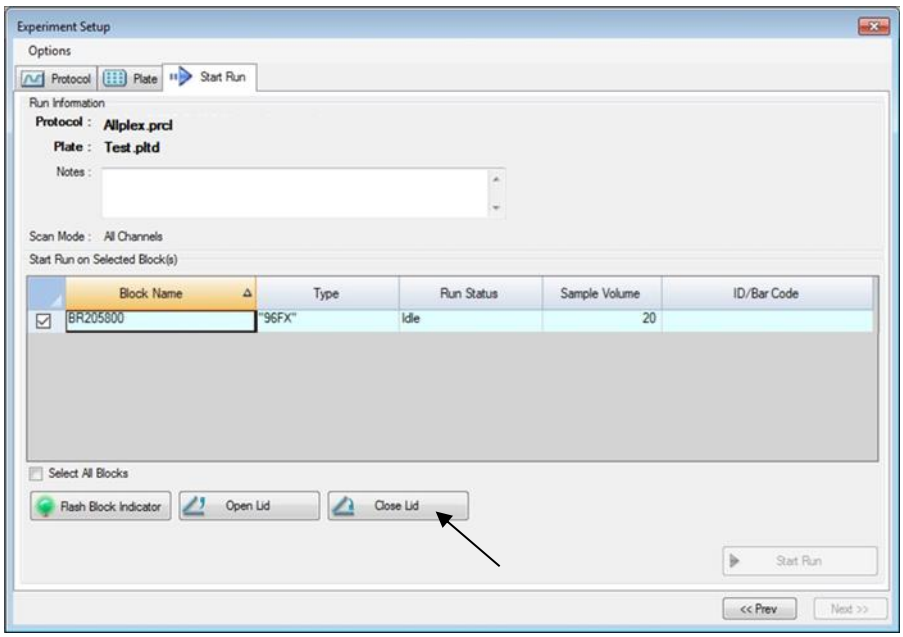


Fig. 8. **Close Lid**

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 of all detection steps of amplification curves from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep3", "QuantStep4" and "QuantStep5" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantitation"** tab to see the amplification curve results.

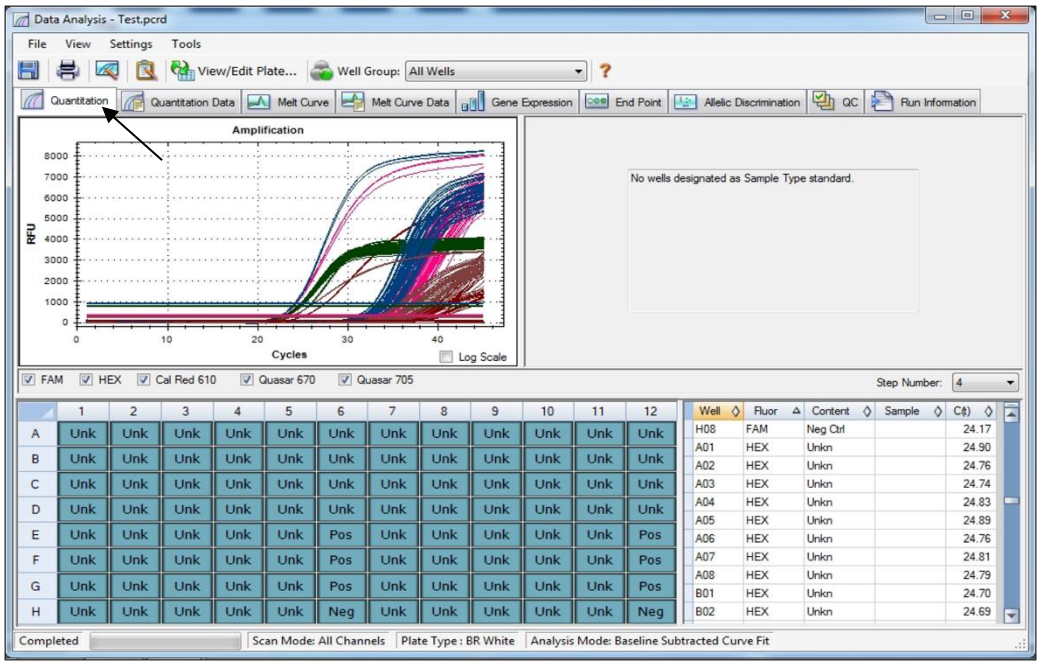


Fig. 9. **Amplification curve results**

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

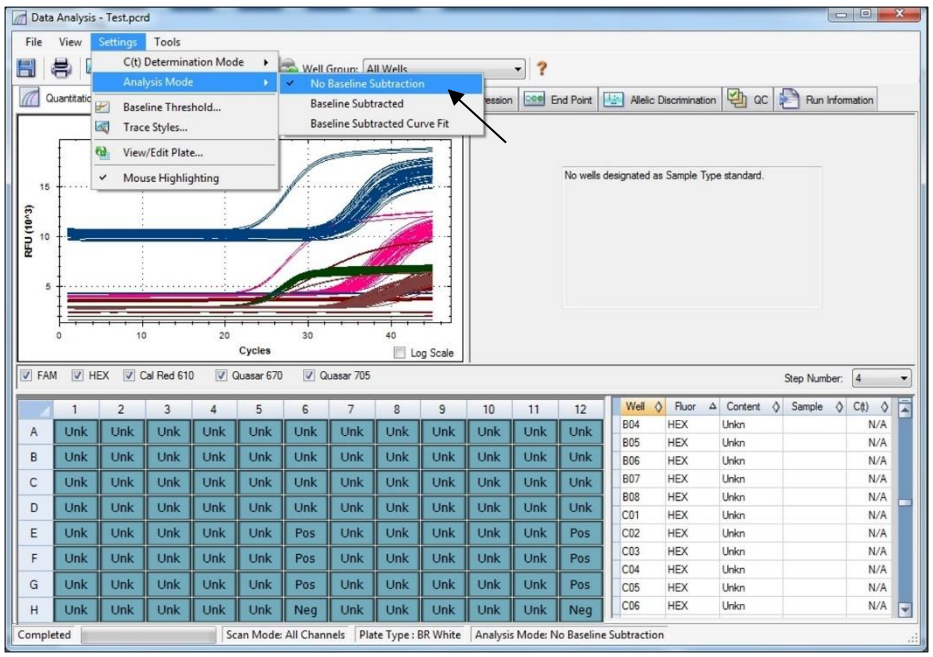


Fig. 10. **No Baseline Subtraction**

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

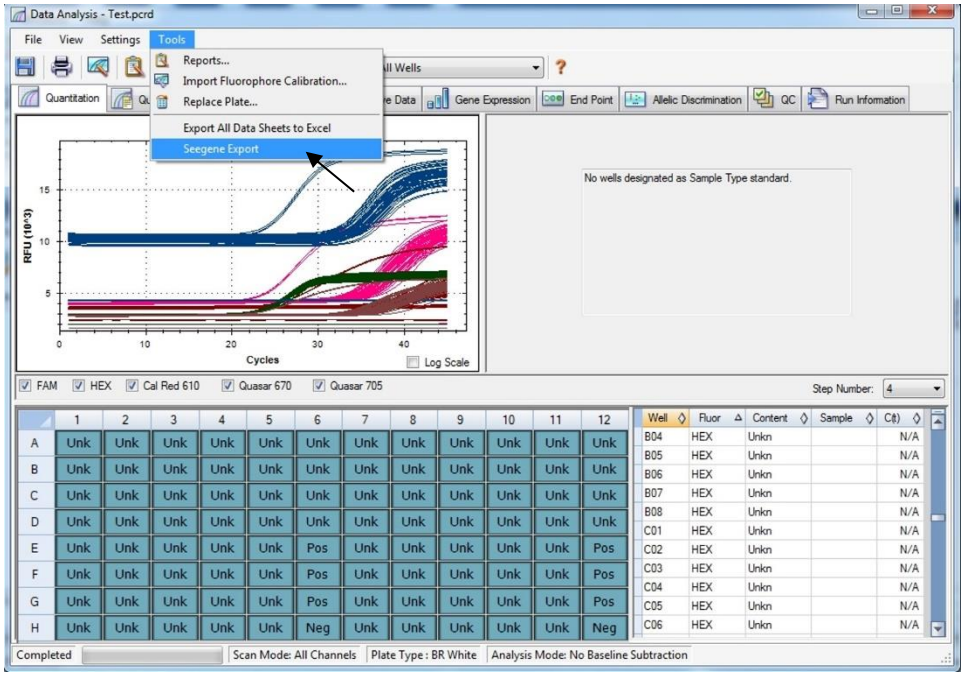


Fig. 11. **Seegene Export**

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

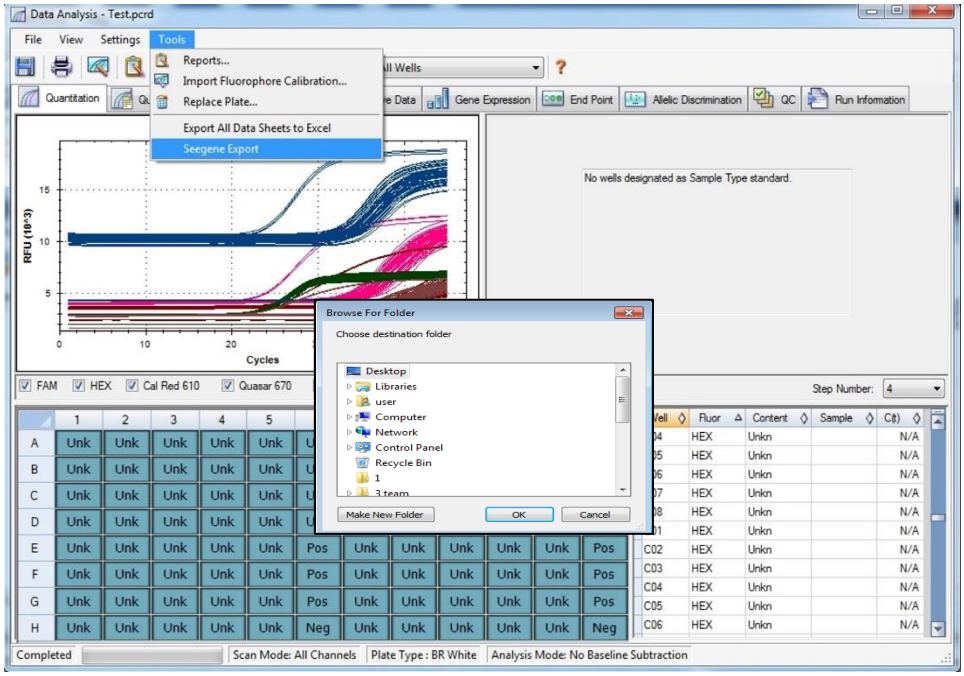


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

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

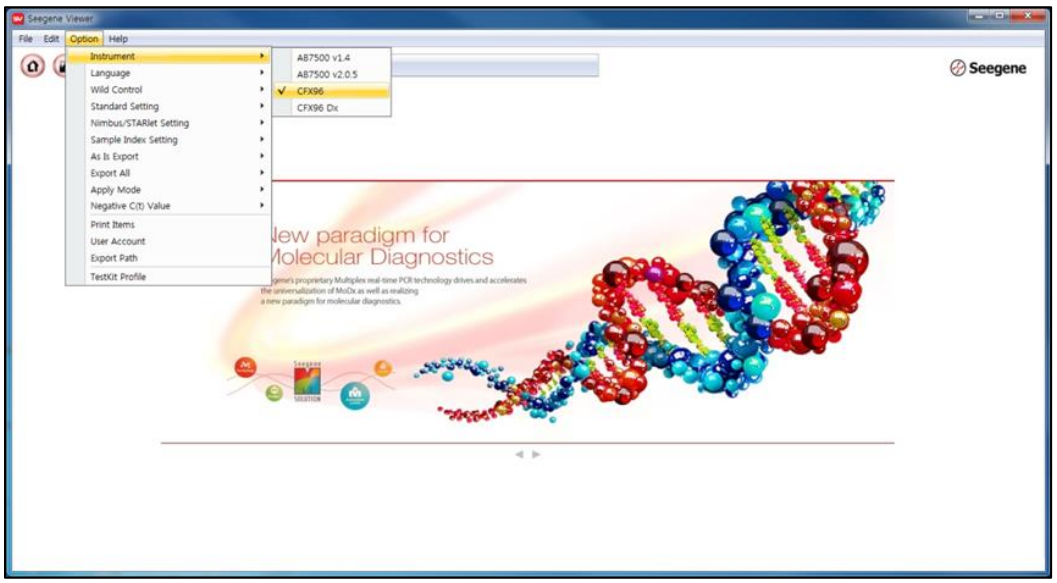


Fig. 13. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep3", open the results file, and select the test kit from the **"PRODUCT"** menu.

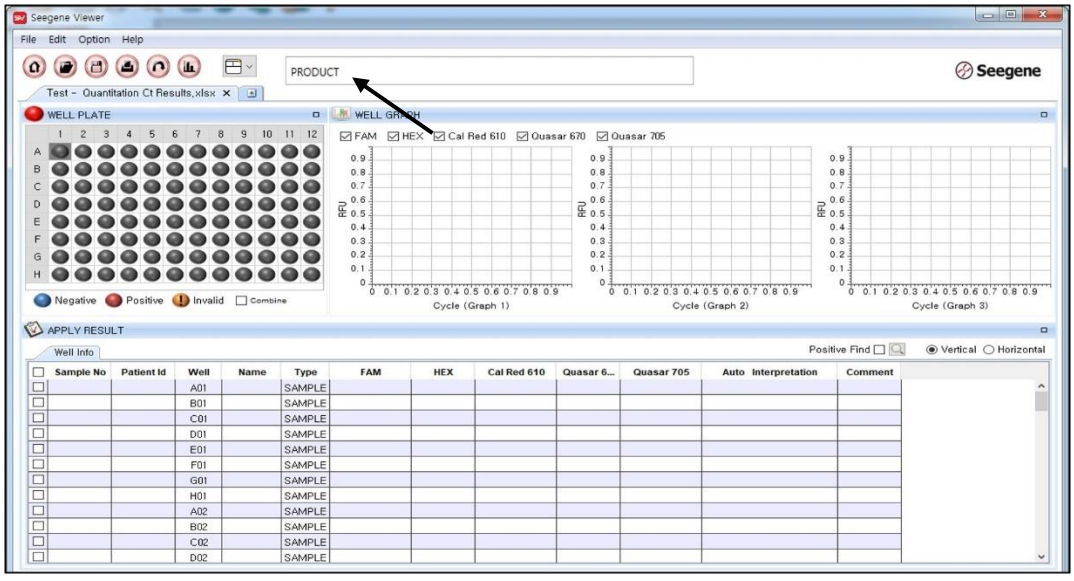


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

Fig. 15. **Test result on Seegene Viewer**

4) Validation Criteria of Control Results

a. Valid Assay Run

To check the validation 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 (Ct)			HEX (Ct)			Cal Red 610 (Ct)			Quasar 670 (Ct)			Quasar 705 (Ct)			Auto Interpretation
	66	45	58	51	59	16	33	39	52	IC	35	18	56	68	31	
Positive Control 1	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	Positive Control(+)
Positive Control 2	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	Positive Control(+)
Positive Control 3	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	Positive Control(+)
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control(-)

b. Invalid Assay Run

In case of a validation failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

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

2.1 Real-time PCR Instrument Setup

Note: CFX96™ Dx System (Bio-Rad) experiment setup can be divided into 3 steps: Protocol Setup, Plate Setup, and Start Run.

A. Protocol Setup

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

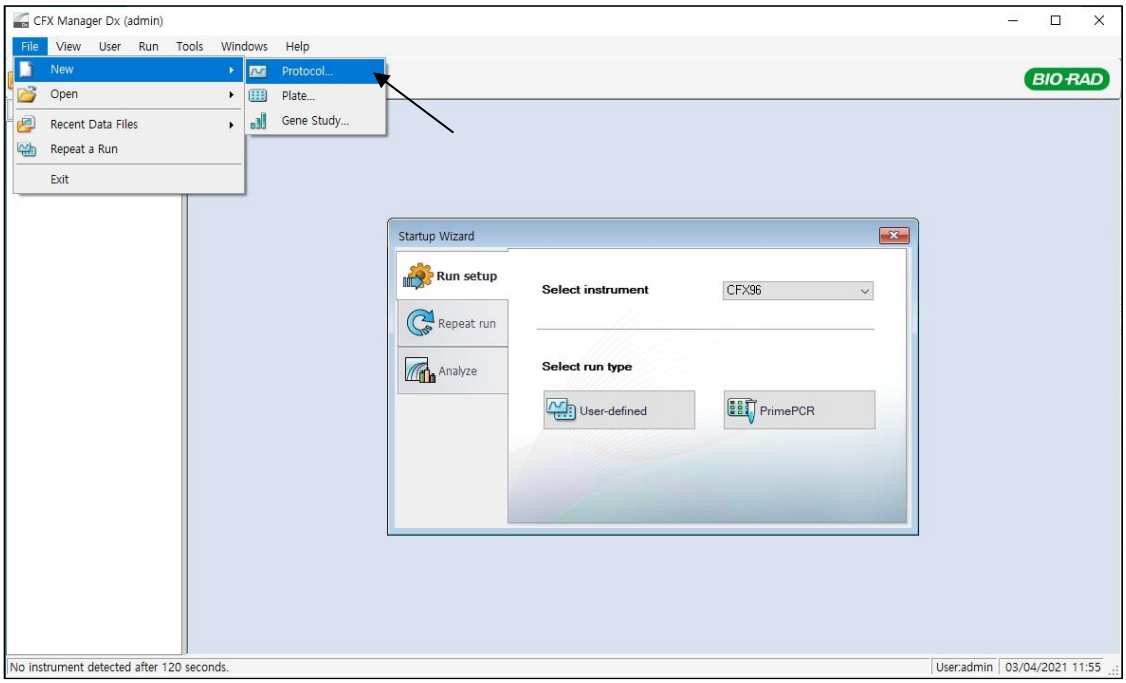


Fig. 1. **Protocol Setup**

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

Step	No. of cycles	Temperature	Duration
1	1	95°C	15 min
2	45	95°C	3 sec
3*		60°C	10 sec
4*		72°C	10 sec
5*		83°C	5 sec

Note*: Plate Read at Step 3, 4 and 5. Fluorescence is detected at 60°C, 72°C and 83°C.

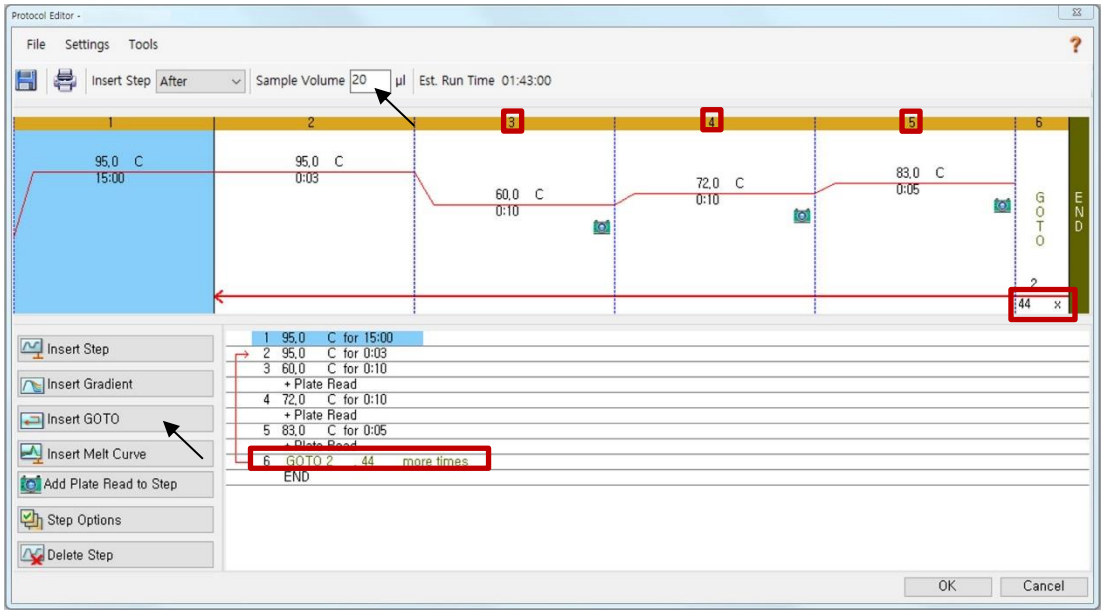


Fig. 2**. Protocol Editor**

Note: Click the "**Insert GOTO**" and type in "**GOTO 2, 44 more times**" at Step 6.

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.

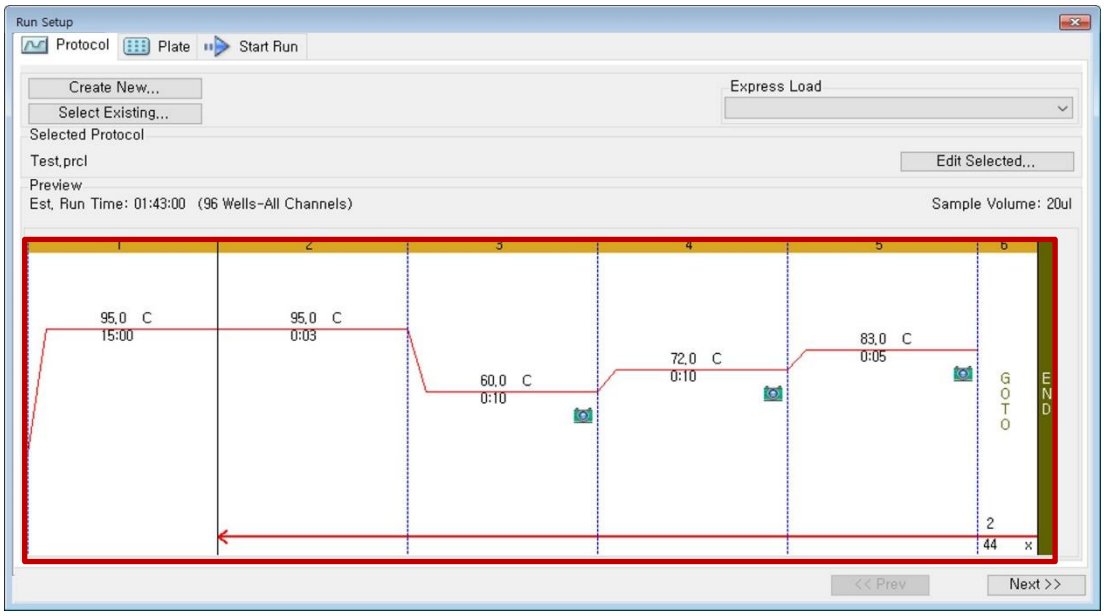


Fig. 3**. Run Setup: Protocol**

B. Plate Setup

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

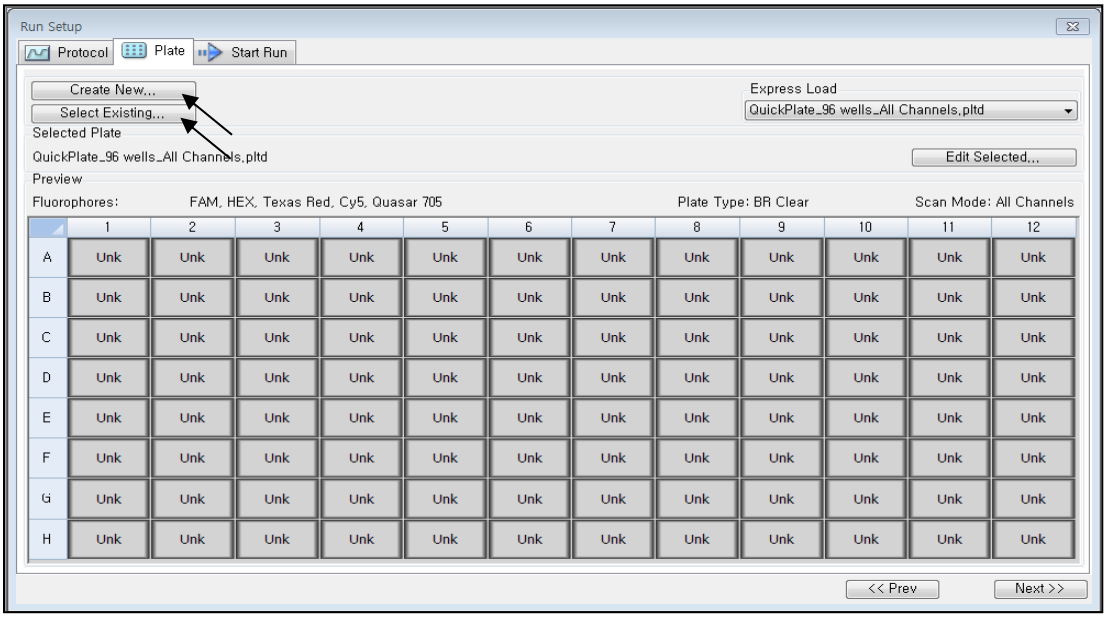


Fig. 4. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM, HEX, Cal Red 610, Quasar 670, Quasar 705***) that will be used and click **"OK"**.

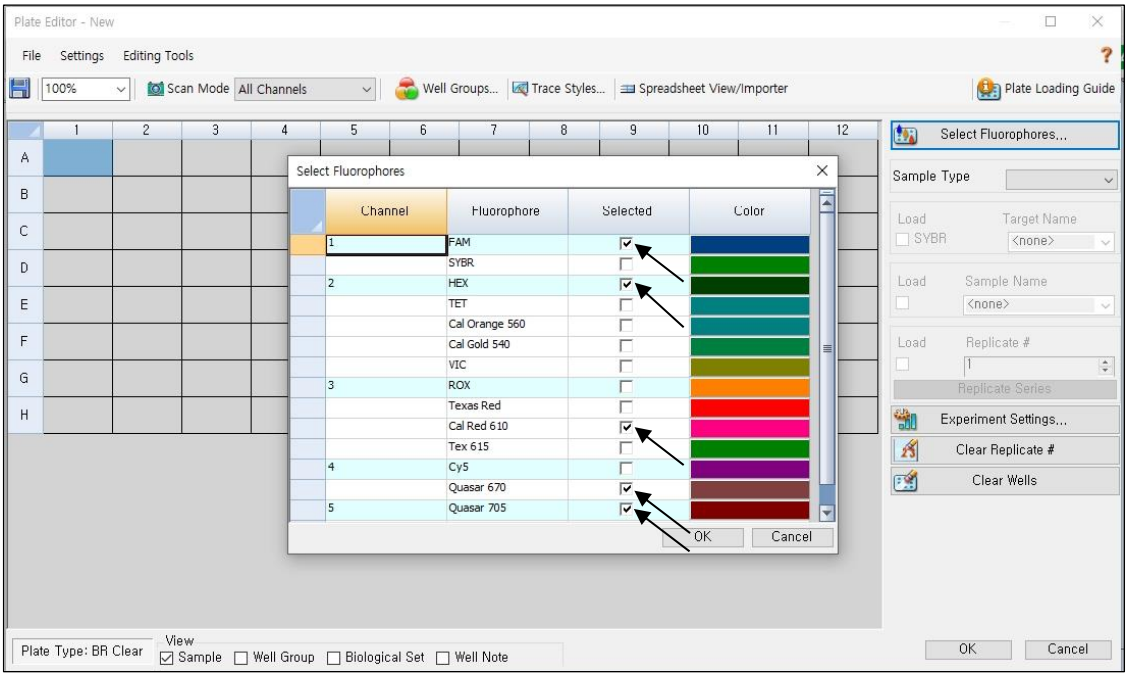


Fig. 5. **"Select Fluorophores"** (***FAM, HEX, Cal Red 610, Quasar 670*** and ***Quasar 705***)

3) 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***

4) Click on the appropriate checkboxes (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670*** and ***Quasar 705***) to specify the fluorophores to be detected in the selected wells.

5) Type in "**Sample Name"** and **PC (PC1, PC2** and **PC3)**, and then press enter key.

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

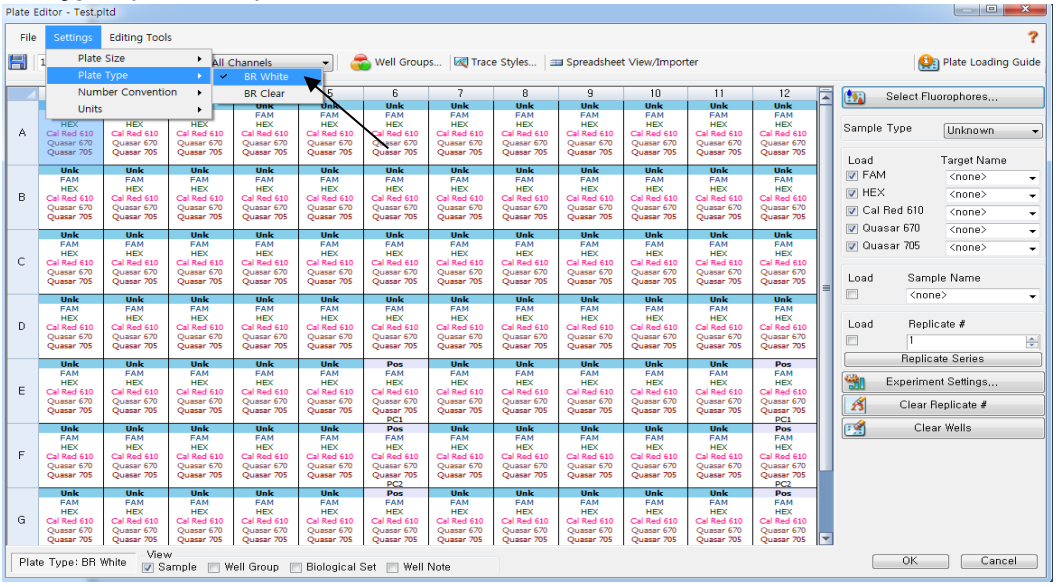


Fig. 6**. Plate Setup**

7) Click **"OK"** to save the new plate.

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

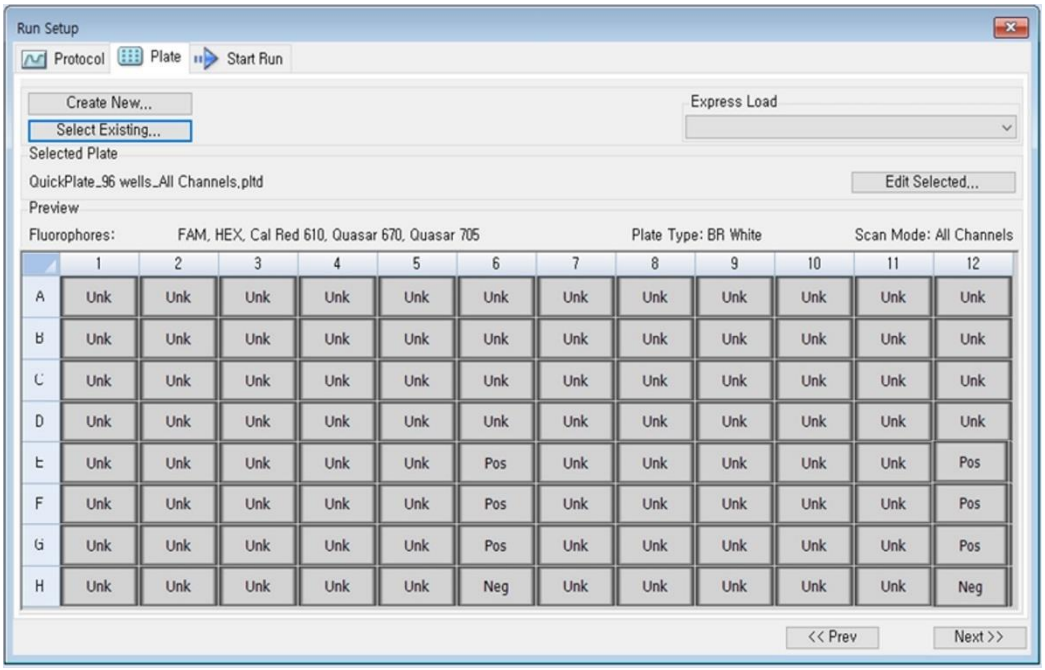


Fig. 7**. Run Setup: Plate**

9) Click **"Next"** to start run.

C. Start Run

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

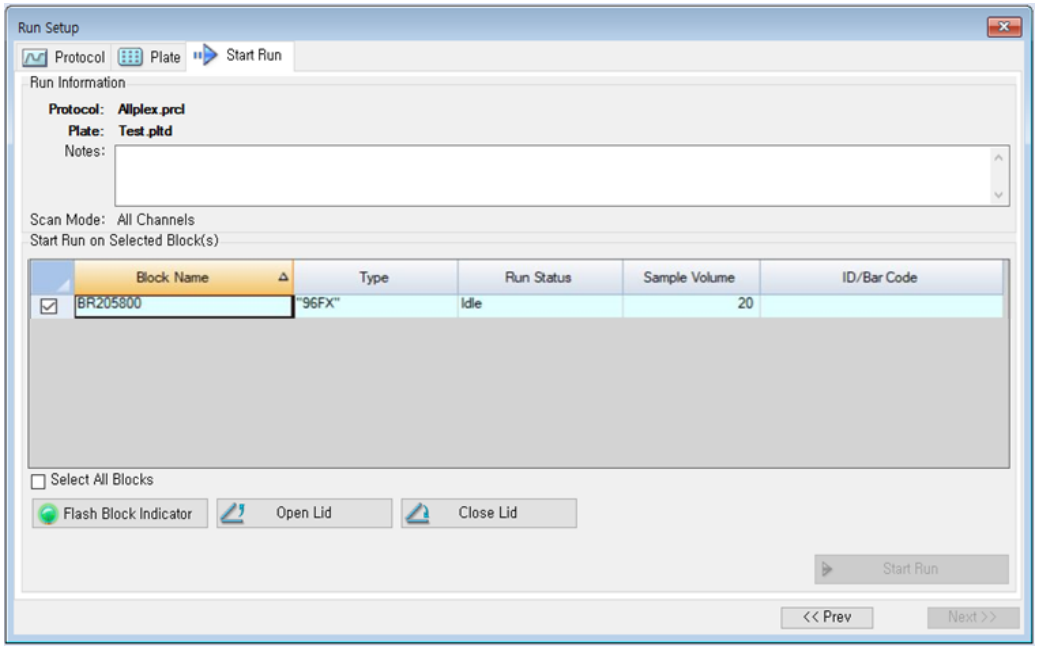


Fig. 8. **Close Lid**

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.

2.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.
2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep3", "QuantStep4" and "QuantStep5" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantification"** tab to see the amplification curve results.

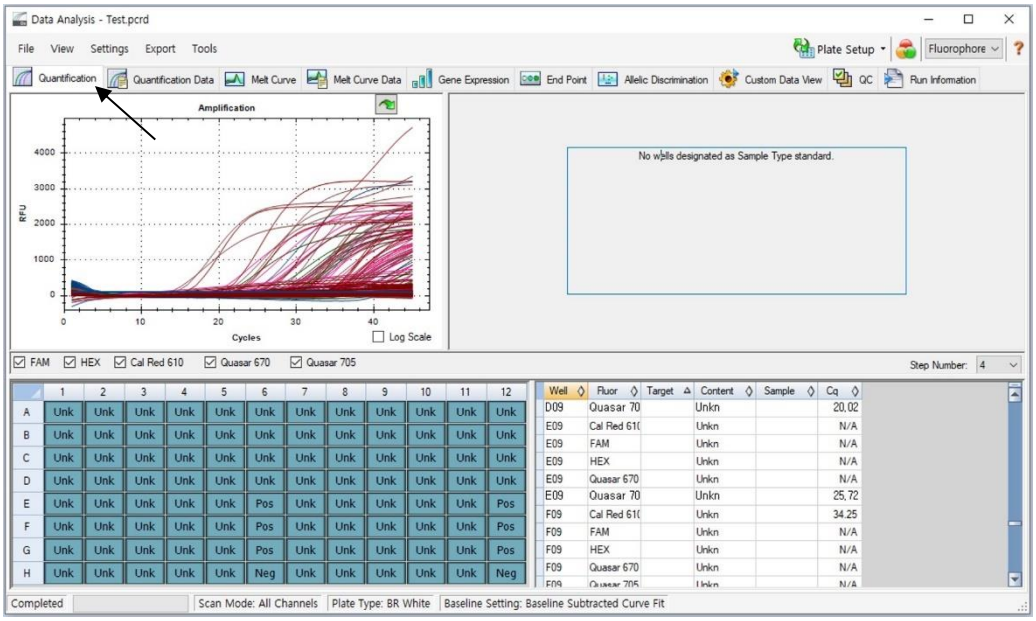


Fig. 9. **Amplification curve results**

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

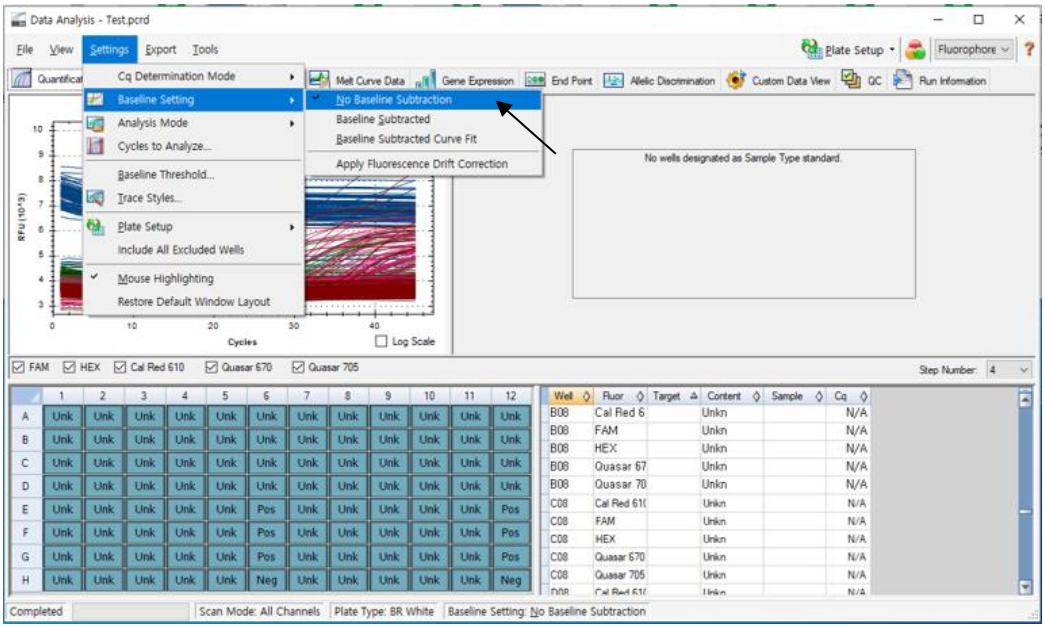


Fig. 10. **No Baseline Subtraction**

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

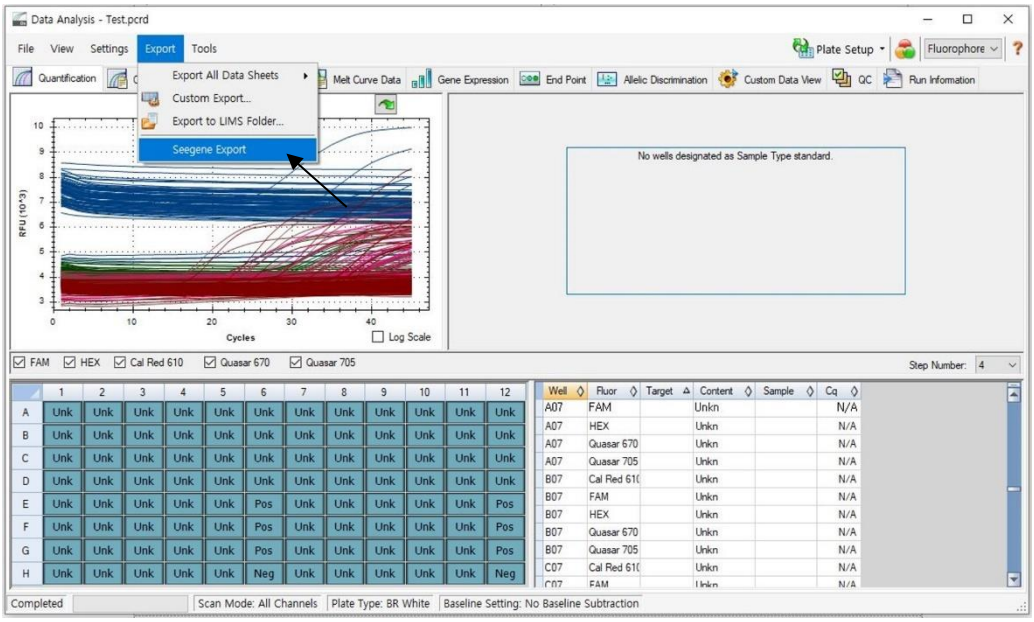


Fig. 11. **Seegene Export**

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

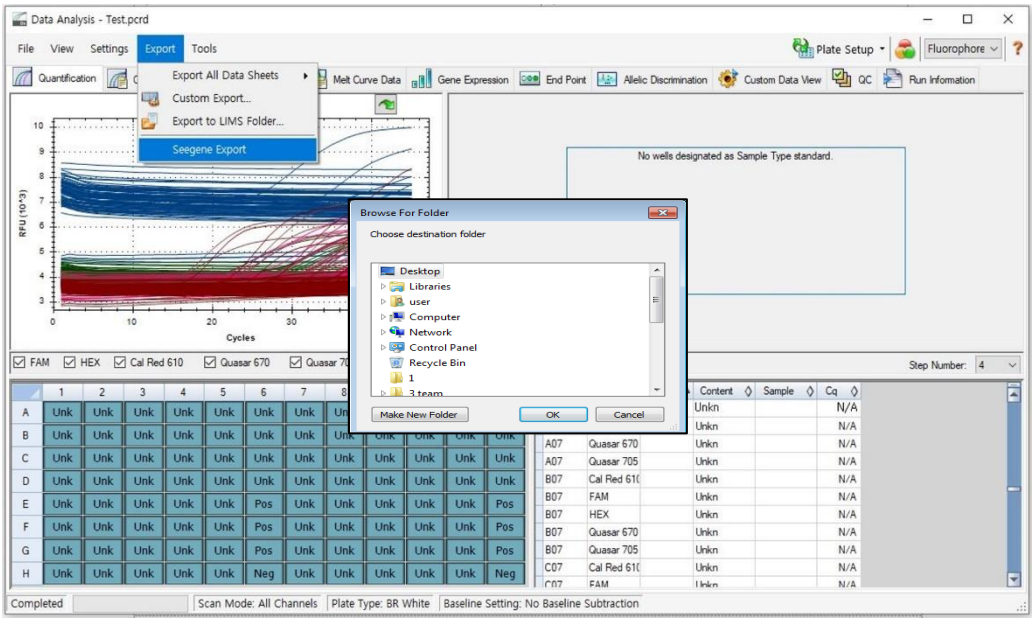


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

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

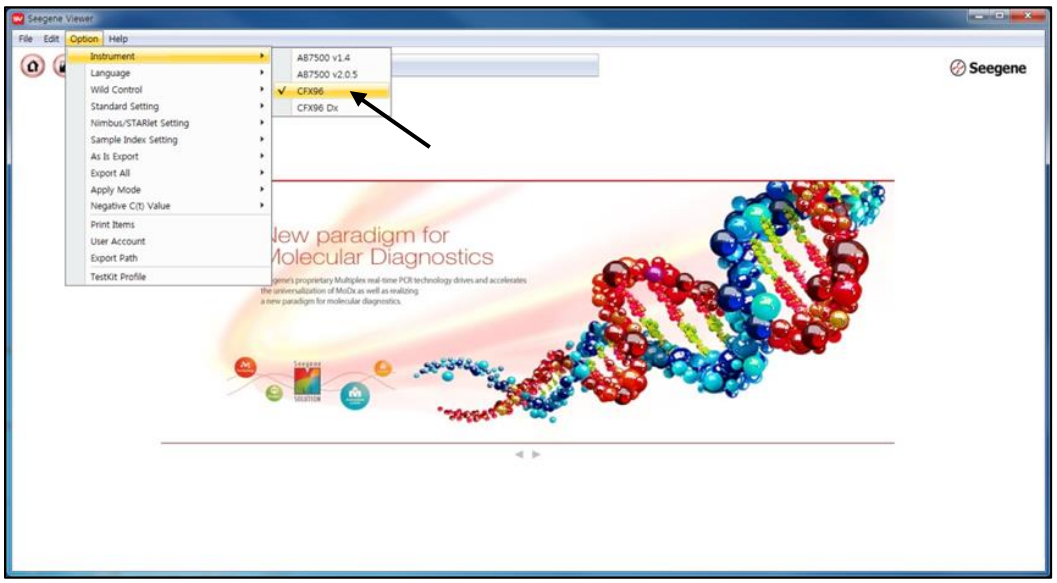


Fig. 13. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep3", open the results file, and select the test kit from the **"PRODUCT"** menu.

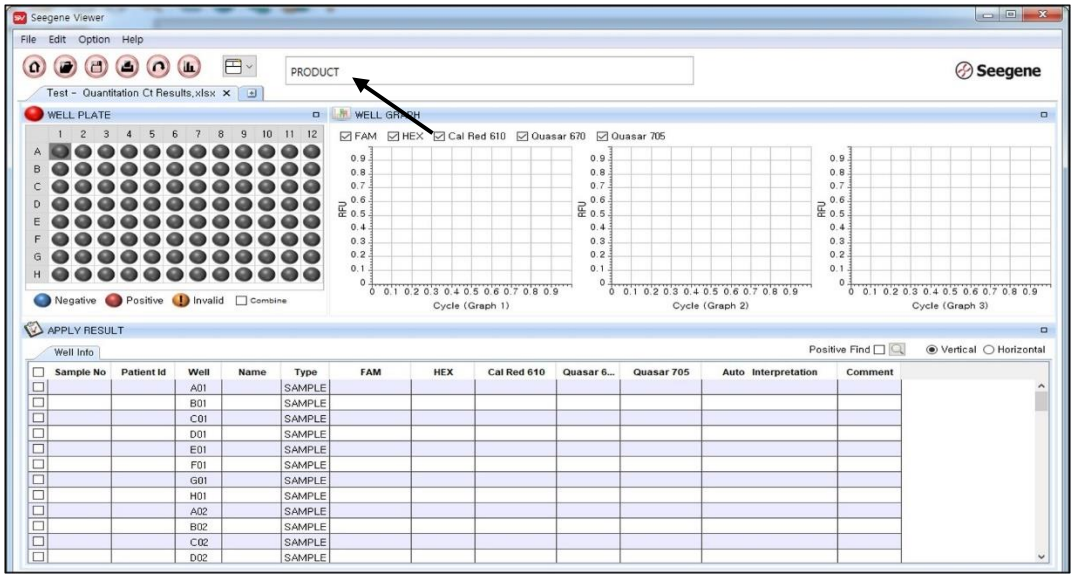


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

Fig. 15. **Test result on Seegene Viewer**

4) Validation Criteria of Control Results

a. Valid Assay Run

To check the validation 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 (Ct)			HEX (Ct)			Cal Red 610 (Ct)			Quasar 670 (Ct)			Quasar 705 (Ct)			Auto Interpretation
	66	45	58	51	59	16	33	39	52	IC	35	18	56	68	31	
Positive Control 1	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	Positive Control(+)
Positive Control 2	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	Positive Control(+)
Positive Control 3	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	-	-	15≤Ct≤31	Positive Control(+)
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control(-)

b. Invalid Assay Run

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

RESULTS

1. Analyte Information

Fluorophores	Analytes		
	Graph 1	Graph 2	Graph 3
FAM	HPV66	HPV45	HPV58
HEX	HPV51	HPV59	HPV16
Cal Red 610	HPV33	HPV39	HPV52
Quasar 670	IC	HPV35	HPV18
Quasar 705	HPV56	HPV68	HPV31

2. Interpretation of Results

Analytes	C_t value	Result
Targets	≤ 43	Detected (+)
	> 43 or N/A	Not detected (-)
IC	≤ 43	Detected (+)
	> 43 or N/A	Not detected (-)

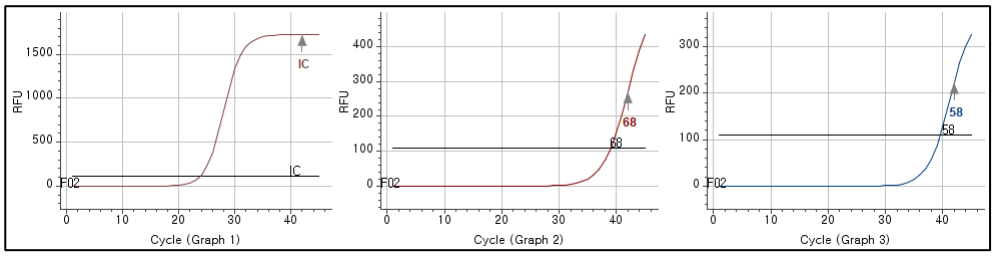
Target Result*	IC Result*	Overall Interpretation
+	+	**Target Nucleic acid, detected** - Target HPV type identification
+	-	**Target Nucleic acid, detected**** - Target HPV type identification - Additional HPV genotypes which may be present were not detected.
-	+	**Target Nucleic acid, not detected**
-	-	**Invalid** - Negative IC signal suggests inadequate specimen collection, processing or the presence of inhibitors. - Repeat the test from the step of nucleic acid extraction using another aliquot of the original specimen.

* Internal Control or any other signals are not observed: see TROUBLESHOOTINGS.

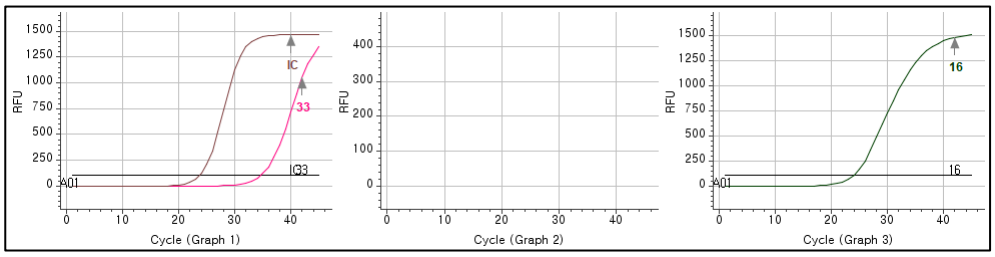
** Internal Control signal could be reduced or absent due to high titer of pathogens.

3. Application to Clinical Samples

Clinical Sample 1



Clinical Sample 2



Seegene Viewer Result (Ct)																		
Sample	**Auto Interpretation**	**Remark**	**Quasar 670**	**FAM**			**HEX**			**Cal Red 610**			**Quasar 670**		**Quasar 705**		
1	High-risk HPV	58,68	**IC**	**66**	**45**	**58**	**51**	**59**	**16**	**33**	**39**	**52**	**35**	**18**	**56**	**68**	**31**
			23.7	N/A	N/A	39.58	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	39.05	N/A
2	High-risk HPV	16,33	**IC**	**66**	**45**	**58**	**51**	**59**	**16**	**33**	**39**	**52**	**35**	**18**	**56**	**68**	**31**
			23.79	N/A	N/A	N/A	N/A	N/A	24.04	34.78	N/A	N/A	N/A	N/A	N/A	N/A	N/A

TROUBLESHOOTINGS

Allplex™ HPV HR Detection		
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 the correct settings.
	Incorrect storage or expiration of the test kit	Please check the storage conditions (See page 10) and the expiration date (refer to label) of the test kit and use a new kit if necessary.
No Internal Control signal	High load of pathogen's nucleic acid	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 observe IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the extracted nucleic acid (1/2~1/5) in RNase-free water and repeat the test from PCR step. If the same result is shown, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Incorrect specimen collection	If both target and IC signal were not observed that means specimen collected inappropriately. Recollect the specimen.
Putative false positive or target signal(s) 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.

Allplex™ HPV HR Detection		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative False negative or no signal observed in Positive Control	Cross-contamination between PC1, 2 and 3	Restart from extraction step or restart from Real-time PCR step.
	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 correct 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 (1/3~1/10) in saline buffer and repeat the test from extraction step.
	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect programming	Repeat the PCR with corrected setting.
	Incorrect PCR mixture	Confirm that all components are added to the reaction mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spin down before use.
	Leaving reagents at room temperature for a long time or incorrect storage condition	Please check the storage condition and the expiry date (see the kit label) of the reagents and use a new kit if necessary.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Spin down the PCR tube before run.

PERFORMANCE

1. Analytical Specificity

The high specificity of Allplex™ HPV HR Detection is ensured by the oligos designed specifically for the targets of interest. Allplex™ HPV HR Detection was tested for cross-reactivity to 105 different pathogens, and PCR amplification and detection were only identified for the specified targets.

No.	Organism	Source	Isolate No.	Result†
1	*Acinetobacter baumannii*	ZMC	0801597	Not detected
2	*Acinetobacter lwoffii*	ZMC	0801909	Not detected
3	Adenovirus Type 1	ZMC	0810050CF	Not detected
4	Adenovirus type 18	KBPV	KBPV-VR-4D	Not detected
5	Adenovirus type 23	KBPV	KBPV-VR-5D	Not detected
6	Adenovirus Type 40	ZMC	0810084CF	Not detected
7	*Bacteroides fragilis*	ZMC	0801583	Not detected
8	*Bifidobacterium longum*	ZMC	0804047	Not detected
9	*Candida albicans*	ZMC	0801504	Not detected
10	*Chlamydia trachomatis*	ZMC	0801775	Not detected
11	*Clostridium perfringens* Type A	ZMC	0801585	Not detected
12	*Corynebacterium genitalium*	ZMC	0804108	Not detected
13	*Cytomegalovirus* (CMV) (Strain: AD-169)	ZMC	0810003CF	Not detected
14	*Enterobacter cloacae*	ZMC	0801597	Not detected
15	*Enterococcus faecalis*	ZMC	0801637	Not detected
16	*Escherichia coli*	ZMC	0801517	Not detected
17	*Fusobacterium nucleatum*	ZMC	0801911	Not detected
18	*Gardnerella vaginalis*	ZMC	0801894	Not detected
19	*Haemophilus ducreyi*	ZMC	0801736	Not detected
20	Herpes Simplex Virus Type 1 (HSV-1) (Strain: MacIntyre)	ZMC	0810005CF	Not detected
21	Herpes Simplex Virus Type 2 (HSV-2) (Strain: MS)	ZMC	0810006CF	Not detected
22	Human Hepatitis B Virus (HBV)	ZMC	NATHBV-0006	Not detected
23	Human immunodeficiency virus (HIV-1)	ATCC	VR-3245SD	Not detected
24	*Klebsiella pneumoniae*	ZMC	0801506	Not detected
25	*Lactobacillus acidophilus*	ZMC	0801540	Not detected

No.	Organism	Source	Isolate No.	Result†
26	*Lactobacillus crispatus*	ZMC	0804143	Not detected
27	*Lactobacillus gasseri*	ZMC	0804327	Not detected
28	*Lactobacillus iners*	ZMC	0804261	Not detected
29	*Lactobacillus jensenii*	ZMC	0804260	Not detected
30	*Mobiluncus curtisii*	ZMC	0804141	Not detected
31	*Mobiluncus mulieris*	ZMC	0804116	Not detected
32	*Mycoplasma hominis*	ZMC	0804011	Not detected
33	*Neisseria gonorrhoeae*	ZMC	0801482	Not detected
34	*Neisseria lactamica*	ZMC	0801752	Not detected
35	*Neisseria meningitidis* Serogroup A	ZMC	0801511	Not detected
36	*Neisseria sicca*	ZMC	0801754	Not detected
37	*Peptostreptococcus anaerobius*	ZMC	0804012	Not detected
38	*Prevotella melaninogenica*	ZMC	0804292	Not detected
39	*Proteus mirabilis*	ZMC	0804544	Not detected
40	*Pseudomonas aeruginosa*	ZMC	0801519	Not detected
41	*Pseudomonas fluorescens*	ZMC	0804248	Not detected
42	*Serratia marcescens*	ZMC	0801723	Not detected
43	Simian Virus 40 (SV40)	ATCC	VRMC-2	Not detected
44	Methicillin-resistant *Staphylococcus aureus* (MRSA)	ZMC	0801638	Not detected
45	Methicillin-resistant *Staphylococcus epidermidis* (MRSE)	ZMC	0801651	Not detected
46	*Streptococcus agalactiae*	ZMC	0801545	Not detected
47	*Streptococcus mitis*	ZMC	0801695	Not detected
48	*Streptococcus pyogenes*	ZMC	0801512	Not detected
49	Syphilis (*Treponema pallidum*)	ZMC	KZMC002	Not detected
50	*Trichomonas vaginalis*	ZMC	0801805	Not detected
51	*Ureaplasma urealyticum*	NCTC	10177	Not detected
52	HPV1	Cloned DNA		Not detected
53	HPV2	Cloned DNA		Not detected
54	HPV3	Korean isolate		Not detected
55	HPV4	Cloned DNA		Not detected
56	HPV5	Cloned DNA		Not detected
57	HPV8	Cloned DNA		Not detected
58	HPV10	Korean isolate		Not detected

No.	Organism	Source	Isolate No.	Result†
59	HPV13	Cloned DNA		Not detected
60	HPV27	Korean isolate		Not detected
61	HPV30	Cloned DNA		Not detected
62	HPV32	Korean isolate		Not detected
63	HPV34	Korean isolate		Not detected
64	HPV55	Korean isolate		Not detected
65	HPV57	Korean isolate		Not detected
66	HPV62	Korean isolate		Not detected
67	HPV67	Korean isolate		Not detected
68	HPV71	Korean isolate		Not detected
69	HPV72	Korean isolate		Not detected
70	HPV74	Korean isolate		Not detected
71	HPV81	Korean isolate		Not detected
72	HPV83	Cloned DNA		Not detected
73	HPV84	Korean isolate		Not detected
74	HPV85	Cloned DNA		Not detected
75	HPV102	Cloned DNA		Not detected
76	SiHa (HPV16 positive)	KCLB	30035	HPV16 detected
77	HeLa (HPV18 positive)	KCLB	10002	HPV18 detected
78	HPV16	NIBSC	06/202	HPV16 detected
79	HPV18	NIBSC	06/206	HPV18 detected
80	HPV31	NIBSC	14/258	HPV31 detected
81	HPV33	NIBSC	14/260	HPV33 detected
82	HPV35	Korean isolate		HPV35 detected
83	HPV39	Korean isolate		HPV39 detected
84	HPV45	NIBSC	14/104	HPV45 detected
85	HPV51	Korean isolate		HPV51 detected
86	HPV52	NIBSC	14/262	HPV52 detected
87	HPV56	Korean isolate		HPV56 detected
88	HPV58	NIBSC	14/264	HPV58 detected
89	HPV59	Korean isolate		HPV59 detected
90	HPV66	Korean isolate		HPV66 detected
91	HPV68	Korean isolate		HPV68 detected
92	HPV6	NIBSC	14/256	Not detected

No.	Organism	Source	Isolate No.	Result†
93	HPV11	NIBSC	14/100	Not detected
94	HPV26	Korean isolate		Not detected
95	HPV40	Korean isolate		Not detected
96	HPV42	Korean isolate		Not detected
97	HPV43	Korean isolate		Not detected
98	HPV44	Korean isolate		Not detected
99	HPV53	Korean isolate		Not detected
100	HPV54	Korean isolate		Not detected
101	HPV61	Korean isolate		Not detected
102	HPV69	Korean isolate		Not detected
103	HPV70	Korean isolate		Not detected
104	HPV73	Korean isolate		Not detected
105	HPV82	Korean isolate		Not detected

† Specificity tests were repeated 3 times.

※ ATCC: American Type Culture Collection
KBPV: Korea Bank for Pathogenic Viruses
ZMC: ZeptoMetrix Corporation
NCTC: National Collection of Type Culture
NIBSC: National Institute for Biological Standards and Control

2. Analytical Sensitivity

In order to determine the limit of detection (LoD) of Allplex™ HPV HR Detection, pDNA for traget 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 2 types of cell lines for target 16 and 18 were serially diluted into pooled HPV negative cervical specimens collected in ThinPrep solution. Nucleic acids were extracted using Microlab NIMBUS IVD (STARMag 96 X 4 Universal Cartridge Kit). The LoD for each target was estimated by probit analysis using software (MedCalc V20.015).

2-1. Limit of Detection: HPV Cell Lines

Target	Limit of Detection (cells/mL)
SiHa (HPV16)	88.9
HeLa (HPV18)	45.2

2-2. Limit of Detection: HPV pDNA

Target	Limit of Detection (copies/mL)
HPV35	3556
HPV39	2515
HPV51	3142
HPV56	3623
HPV59	3660
HPV66	3941
HPV68	3586

Target	Limit of Detection (IU/mL)
HPV16	4134
HPV18	1217
HPV31	3680
HPV33	1616
HPV45	5643
HPV52	2967
HPV58	2263

3. Reproducibility

The reproducibility test was prepared including Moderate positive (3X LoD) and Low positive (1X LoD) samples. At each testing site, the kit was tested for 5 days, 2 runs per day by 2 different experimenters and triplicate of each target. The positive rates were observed for each target for reproducibility study: 100.0% for Moderate positive samples, ≥95% for Low positive samples. The reproducibility of Allplex™ HPV HR Detection was evaluated between runs, sites and product lots. Positive rates for all concentrations met criteria, and CV values were less than 10 (<10).

The results were satisfied with the criteria set above, thus confirming the reproducible performances of Allplex™ HPV HR Detection.

4. Interfering substances

There were no effects on the results by adding the substance: non-specific detections or inhibitions on target amplification. Based on the results, 7 different types of interfering substances had no effect on Allplex™ HPV HR Detection results.

No.	Interfering Substances	Source	Test Concentration
1	Blood	Human	5% v/v
2	Leukocytes, Sonicated	Lee Biosolutions (Cat.No. 342-10-1)	$1X10^6$ cells/mL
3	Mucin (Mucin from porcine stomach)	Sigma-Aldrich (Cat.No. M1778-10G)	10% v/v
4	Spermicide (Nonoxynol-9)	Abcam (Cat.No. ab143673)	10% w/v
5	Yeast Gard Advanced®	Lake Consumer Products, Inc.	10% w/v
6	Lubricant	Vagisil®	10% w/v
7	Contraceptive pill (Mercilon®)	Alvogen®	10% w/v

5. Clinical performance

[Performance comparison to a CE-IVDD approval comparator]

Allplex™ HPV HR Detection showed an equivalent clinical performance as a primary cervical cancer screening test in comparison to the reference assay in risk stratification for CIN 2+, based on the central pathology review diagnoses of cervical cancer. As per the diagnosis of cervical intraepithelial neoplasms (CIN), relative sensitivity and relative specificity between the test assay and comparator HPV DNA testing should be above 90% and 98%, respectively, which are set based on the equivalency criteria (Arbyn et al., 2015). Allplex™ HPV HR Detection met the designated criteria and showed its clinical validity.

		Histology		
		CIN2+	<CIN2	Total
Allplex™ HPV HR Detection	Positive	314	276	590
	Negative	99	127	226
	Total	413	403	816

		Histology		
		CIN2+	<CIN2	Total
CE-IVDD approval comparator	Positive	318	274	592
	Negative	95	129	224
	Total	413	403	816

Relative sensitivity (Allplex™ vs Comparator)	98.74%
Relative specificity (Allplex™ vs Comparator)	98.45%

[Clinical equivalence of Allplex™ HPV HR Detection between cervical and self-collected vaginal specimens]

Allplex™ HPV HR Detection showed an equivalent clinical validity in self-collected vaginal specimens. The paired specimens of 143 cervical specimens and 143 self-collected vaginal specimens were included in the clinical performance. Allplex™ HPV HR Detection showed an overall percent agreement (OPA) of above 95% and the agreement was equivalent between the paired cervical specimens and self-collected vaginal specimens, suggesting the equivalent clinical performance between the paired specimens.

	Cervical specimens (Allplex™ HPV HR vs CE-IVDD approved comparator)				Self- collected vaginal specimens (Allplex™ HPV HR vs CE-IVDD approved comparator)			
	OPA (%)	95% CI	Kappa	95% CI	OPA (%)	95% CI	Kappa	95% CI
HPV16	98.60 (141/143)	95.04 to 99.83	0.868	0.687 to 1.000	97.90 (140/143)	93.99 to 99.57	0.812	0.605 to 1.000
HPV18	100 (143/143)	97.45 to 100.00	1.000	1.000 to 1.000	100 (143/143)	97.45 to 100.00	1.000	1.000 to 1.000
HPV16 or HPV18	99.30 (142/143)	96.17 to 99.83	0.937	0.816 to 1.000	98.60 (141/143)	95.04 to 99.83	0.881	0.719 to 1.000
Other HPV	95.10 (136/143)	90.17 to 98.01	0.876	0.786 to 0.965	95.10 (136/143)	90.17 to 98.01	0.876	0.787 to 0.965
HR-HPV Positive	96.50 (138/143)	92.03 to 98.86	0.916	0.845 to 0.988	95.80 (137/143)	91.09 to 98.44	0.889	0.820 to 0.978

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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
ENZYME	Enzyme mix
BUFFER	Buffer
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
	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™ HPV Series		
HP10371Z	**Allplex™ HPV HR Detection**	**25 rxns**
HP10370X	**Allplex™ HPV HR Detection**	**100 rxns**
HP10376L	**Allplex™ HPV HR Detection**	**100 rxns x 8 kits**
HP10373Z	Allplex™ HPV28 Detection	25 rxns
HP10372X	Allplex™ HPV28 Detection	100 rxns
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
SG71101	Seegene STARlet 96MPH	EA
SG72100	AIOS	EA
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384T / 1box
EX00036P	STARMag™ S96H N Kit	480T / 1box
EX00037P		960T / 1box
SG71100	SEEPREP32	EA
EX00009P	STARMag 96 ProPrep (Plate Type)	96T / 1box
EX00009T	STARMag 96 ProPrep (Tube Type)	96T / 1box