For Professional Use Only

Anyplex™ II

HPV HR Detection

(Cat. No. HP7E00X, HP10380Z)

Anyplex™ II 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 liquid-based cytology, cervical swab 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 **HP7E00X** Σ 100 REF **HP10380Z** Σ 25

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.
- If this product is used with **Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet,** it provides a maximum of 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: cervical swab, self-collected vaginal specimen and liquid-based cytology specimens**. This test has not been validated for any other types of specimens.
- **Store DNA samples at -70℃ 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 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. The use of sterile 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 prevents any potential splashing or cross-contamination of specimens during preparations.
- Please be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive control. To prevent the contamination of reagents, the 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 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 13 months at ≤-20℃ from the date of manufacture. 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.
- This kit is intended to aid in the differential diagnosis of target pathogen infections;
  Human papillomaviruses.

INTENDED USE

The Anyplex™ II HPV HR Detection is a qualitative *in vitro* test for the detection of 14 high risk HPV types in liquid-based cytology, cervical swab and self-collected vaginal specimens. This assay specifically identifies not only HPV 16 and HPV 18 but also other 12 individual high risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) at clinically relevant infection levels.

The Anyplex™ II HPV HR Detection is indicated:

a) To screen patients with ASC-US (atypical squamous cells of undetermined significance) cervical cytology results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy.
b) To screen patients with ASC-US cervical cytology results to assess the presence or absence of HPV 16, HPV 18 and other 12 individual high risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).
c) To be used with cervical cytology to adjunctively screen to assess the presence or absence of HPV 16, HPV 18 and other 12 individual high risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).
d) 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.
e) To be used as a primary screening test to assess the presence or absence of HPV 16, HPV 18 and other 12 individual high risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

The result from the Anyplex™ II 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

The Anyplex™ II HPV HR Detection represents Seegene's proprietary technologies and is based on a TOCE™ technology which makes it possible to detect multi-pathogens in a single fluorescence channel on real-time PCR instruments.

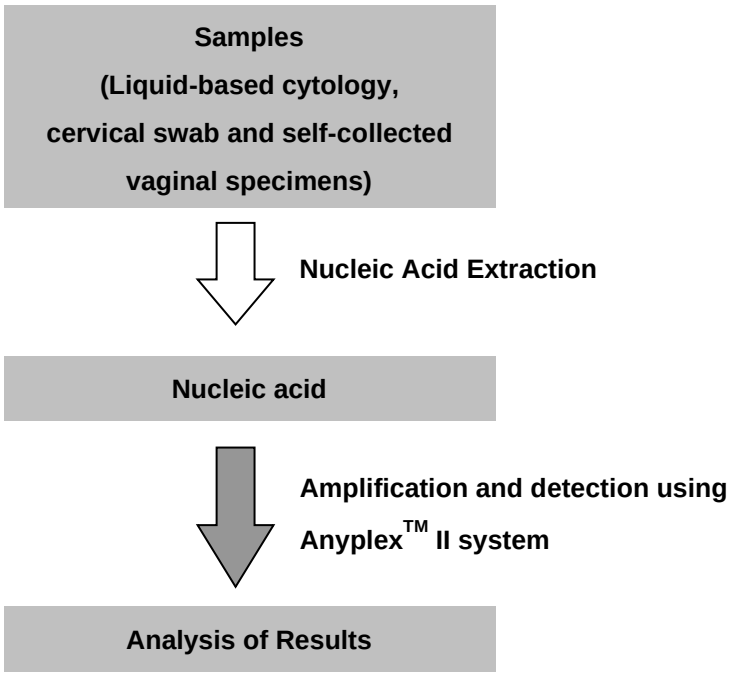
In current melting curve analysis, temperature differences are often observed among DNAs that have high sequence variation, resulting in issues the field of clinical diagnostics where accurate and reproducible test results are critical. However, TOCE™ technology is designated not to be affected by sequence variations; therefore, it guarantees consistent Tm values.

The Anyplex™ II HPV HR Detection can perform multiplex examination by either End point-CMTA (End point-Catcher Melting Temperature Analysis) or cyclic-CMTA (cyclic-Catcher Melting Temperature Analysis) method. cyclic-CMTA method which represents a new class of molecular tests can discriminate major pathogen in the co-infected samples. The Anyplex™ II HPV HR Detection is a multiplex real-time PCR assay that permits the simultaneous amplification, detection and differentiation of target nucleic acids of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) as well as Internal Control (IC).

In PCR, 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. The Anyplex™ II HPV HR Detection uses a human house-keeping gene as an endogenous IC which can ensure purification of DNA, verification of PCR reaction and clarification of cell adequacy from each specimen.

The Uracil-DNA glycosylase (UDG)-dUTP system is employed in the Anyplex™ II HPV HR Detection. 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

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. Eventually the Anyplex™ II HPV HR Detection only specifically detects true HPV and accurately genotypes them. It also contains endogenous Internal Control 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. In addition, high-risk HPV group has been reported to be associated with the development of cervical cancer; especially, HPV16 and HPV18 about 70% of cervical cancer cases. Therefore, early diagnosis using HPV DNA testing is necessary to prevent cancer progression. Anyplex™ II 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 HP7E00X)

Anyplex™ II HPV HR Detection			
Symbols	**Contents**	**Volume**	**Description**
PRIMER	4X HPV HR TOM	500 µL	TOCE Oligo Mix (TOM): - Amplification and detection reagents
PREMIX	EM1	500 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	HPV HR PC1	50 µL	Positive Control(PC) : - Mixture of pathogen clones
CONTROL +	HPV HR PC2	50 µL	Positive Control(PC) : - Mixture of pathogen clones
CONTROL +	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 HP10380Z)

Anyplex™ II HPV HR Detection			
Symbols	**Contents**	**Volume**	**Description**
PRIMER	4X HPV HR TOM	125 µL	TOCE Oligo Mix (TOM): - Amplification and detection reagents
PREMIX	EM1	125 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	HPV HR PC1	50 µL	Positive Control(PC) : - Mixture of pathogen clones
CONTROL +	HPV HR PC2	50 µL	Positive Control(PC) : - Mixture of pathogen clones
CONTROL +	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 the Anyplex™ II HPV HR Detection should be stored at ≤-20℃. 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, 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)
- Proteinase K (For SEEPREP12™, Cat. No.P4850, SIGMA)
- 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 :

Liquid-based cytology specimen
Cervical swab 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

Liquid-based cytology specimen

- Follow the manufacturer's instructions for collecting cervical cell specimens into ThinPrep® (HOLOGIC, USA) or SurePath™ (Becton-Dickinson, USA) or CellPreserv (Kolplast, Brazil) media.

Cervical swab specimen

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

- Cervical swabs can be collected and transported in the following mediums :
  - eNAT® (COPAN, Italia)

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)
  - Qvintip® (Aprovix AB, Sweden)

Self-sampling device	Manufacturer	Cat. No.
Rovers® Evalyn® Brush	Rovers Medical Devices B.V.	380500131
Qvintip®	Aprovix AB	10-002

- 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		Note
		Temp.	Duration*	
Cervical swab	eNAT®	2~8℃ & Room Temperature**	90 days	- Performance may be affected by prolonged storage of specimens. - Specimens should also adhere to local and national instructions for transport of pathogenic material.
Liquid-based cytology	ThinPrep®			
	SurePath™			
	CellPreserv			
Self-collected vaginal specimen	ThinPrep®			

* 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℃.

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.

A. Pre-treatment of Liquid-based cytology specimen

- Equilibrate samples to room temperature (19~25℃).
- Centrifuge 1 mL of liquid-based cytology 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, D) should be resuspended in 1X PBS by vortexing thoroughly to redissolve.

Note: Process pre-treatment step using lysis buffer in extraction kit not 1X PBS 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® and SurePath™ media can be processed without pre-treatment when using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet.

Note: CellPreserv and self-collected vaginal specimen do not require a pre-treatment step.

Note: SurePath™ and CellPreserv have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

Note: CellPreserv has not been validated with STARMag 96 ProPrep C (Plate Type) and STARMag 96 ProPrep C (Tube Type)

- Follow the manufacturer's protocol.

B. Cervical swab specimen

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

C. Manual Nucleic Acid Extraction Kits

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

Extraction Kit	Manufacturer	Cat. No.	Recommended Vol.
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.

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

D. Automated Extraction Systems

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

D-1. Microlab NIMBUS IVD

Note: See Microlab NIMBUS IVD operation manual.

Automated Extraction Systems	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
STARMag 96 X 4 Viral DNA/RNA 200 C Kit**	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

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

****** SurePath™ and CellPreserv have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

D-2. Microlab STARlet IVD

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

Automated Pre-analytic System	Manufacturer	Cat. No.
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: See Microlab STARlet IVD operation manual.

Automated Extraction Systems	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
STARMag 96 X 4 Viral DNA/RNA 200 C Kit**	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

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

****** SurePath™ and CellPreserv have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

D-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
STARMag 96 X 4 Viral DNA/RNA 200 C Kit*	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

***** SurePath™ and CellPreserv have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

D-4. Seegene STARlet

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

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

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

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) 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 used with AIOS, this product can be used for maximum 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 96 X 4 Viral DNA/RNA 200 C Kit*	Seegene	EX00013C	Specimen: 300 μL Elution: 100 μL
STARMag™ S96H N Kit**	Seegene	EX00036P EX00037P	Specimen: 300 μL Elution: 60 μL

***** SurePath™ and CellPreserv have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

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

D-5. SEEPREP12™

Automated Extraction Systems	Manufacturer	Cat. No.	Recommended Vol.
SEEPREP12™	DiaSorin	SPN1200*	-
SEEPREP12™ Viral NA Kit**	DiaSorin	SPN1004*	Specimen: 240 µL Elution: 60 µL

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

** Proteinase K (20 mg/mL) is not included in this kit.

- Add 10 µL of proteinase K (20 mg/mL; purchase separately) to each 1.5 mL sample tubes.
- Transfer 240 µL of specimen to the tube containing 10 µL of proteinase K, mix by flicking the tube gently.
- The cartridge and assembled pump-tip are placed on the instrument.
- Place 1.5 mL elution tube onto the instrument.
- Press **"CONTINUE"** on the first screen to let the instrument initialize.
- Press **"START PROTOCOL"** on the SEEPREP12™ main menu.
- In **Select protocol** menu, press "SPN Viral NA-HT v.2.0".
- In **Select sample volume** menu, press **"250 µL"** and in **Select elution volume**, press **"60 µL"**.

- Follow the onscreen instructions for loading the instrument.
- After all steps are completed, close the lid and start the run.

D-6. NucliSENS® easyMAG®

- Proceed the extraction process using **'generic protocol'**.

Automated Extraction System	Manufacturer	REF	Recommended Vol.
NucliSENS® easyMAG®	bioMérieux	200111	Specimen: 200 μL Magnetic Silica: 50 μL Elution: 100 μL

D-7. SEEPREP32

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

Automated Extraction System	Manufacturer	REF	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
STARMag 96 ProPrep C (Plate Type)*	Seegene	EX00017P	Specimen: 200 μL Elution: 100 μL
STARMag 96 ProPrep C (Tube Type)*	Seegene	EX00017T	Specimen: 200 μL Elution: 100 μL

***** CellPreserv has not been validated with STARMag 96 ProPrep C (Plate Type) and STARMag 96 ProPrep C (Tube Type).

E. Summary

Extraction Method	Applicated sampling device
Microlab NIMBUS IVD / Microlab STARlet IVD / Seegene NIMBUS / Seegene STARlet [8]	eNAT®, ThinPrep®, SurePath™ [1,2], CellPreserv [1]
SEEPREP12™ [3]	eNAT®, ThinPrep® [7], SurePath™
NucliSENS® easyMAG® [4]	eNAT®, ThinPrep® [7], CellPreserv
QIAamp® DNA Mini Kit	eNAT®, ThinPrep®, SurePath™ [5], CellPreserv
Ribo_spin vRD (Viral RNA/DNA Extraction Kit)	eNAT®, ThinPrep®, CellPreserv
SEEPREP32	eNAT®, ThinPrep®, CellPreserv [6]

1. SurePath™ and CellPreserv have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

2. If DNA is extracted from SurePath™ specimens with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet, there is a possibility that the sensitivity could be reduced compared to other extraction methods.

3. Only available for SEEPREP12™ Viral NA Kit.

4. NucliSENS® easyMAG system

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

6. CellPreserv has not been validated with STARMag 96 ProPrep C (Plate Type) and STARMag 96 ProPrep C (Tube Type).

7. ThinPrep® media with self-collected vaginal specimens have not been validated with SEEPREP12™ and NucliSENS®easyMAG®.

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

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

** Optional: AIOS can be used with Seegene STARlet.

3. Preparation for Real-time PCR

Note: When using Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet 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: 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 and Seegene STARlet. Refer to each operation manual.**

A. Prepare PCR Mastermix.

5 μL	4X HPV HR TOM
5 μL	EM1
5 μL	RNase-free Water
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 spin down.

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

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 each HPV HR PC1, PC2 and PC3.

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

Note: Do not label the cap of the reaction tubes as fluorescence is detected through the cap.

- **Positive Control**

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

Each PC includes clones for 5 targets.

Note: To run the Positive Control reaction, prepare three 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 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.**

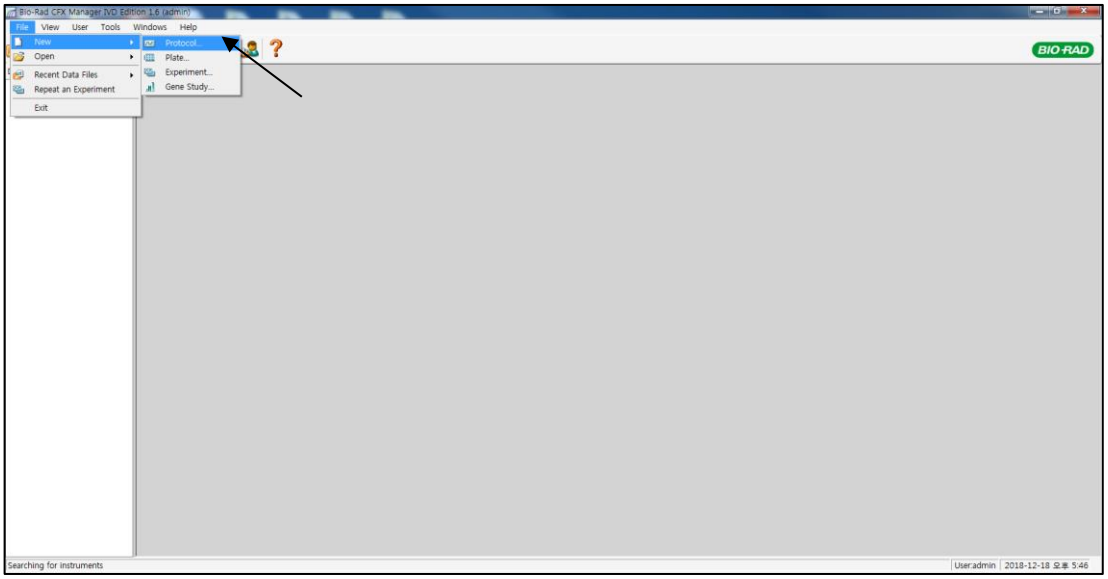


Fig. 1. **Protocol Setup**

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

i) cyclic-CMTA (Melt analysis of three times)

Step	Temperature	Duration	No. of cycles
1	50°C	4 min	
2	95°C	15 min	
3	95°C	30 sec	30
4	60°C	1 min	
5	72°C	30 sec	
6	GOTO 3, 29 more times		
7	55°C	30 sec	
8*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		
9	95°C	30 sec	10
10	60°C	1 min	
11	72°C	30 sec	
12	GOTO 9, 9 more times		
13	55°C	30 sec	
14*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		
15	95°C	30 sec	10
16	60°C	1 min	
17	72°C	30 sec	
18	GOTO 15, 9 more times		
19	55°C	30 sec	
20*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		

***Note: Plate Read at Steps 8, 14 and 20.** Fluorescence is detected at Melting.

ii) End point-CMTA (Melt analysis of one time)

Step	Temperature	Duration	No. of cycles
1	50°C	4 min	
2	95°C	15 min	
3	95°C	30 sec	50
4	60°C	1 min	
5	72°C	30 sec	
6	GOTO 3, 49 more times		
7	55°C	30 sec	
8*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		

***Note: Plate Read at Step 8.** Fluorescence is detected at Melting.

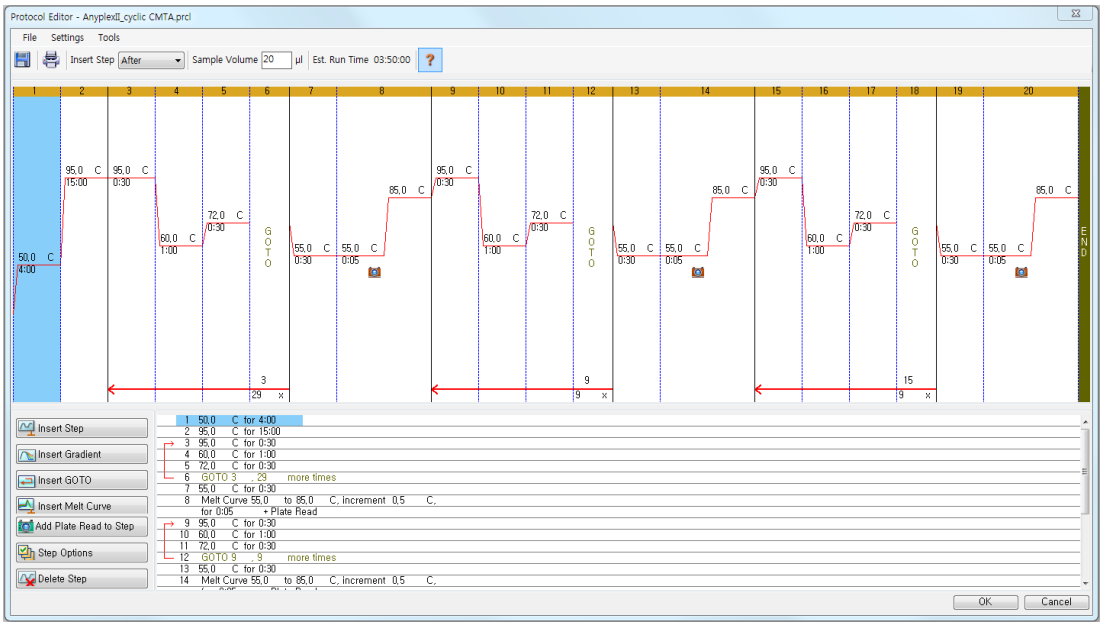


Fig. 2. **Protocol Editor** (cyclic-CMTA)

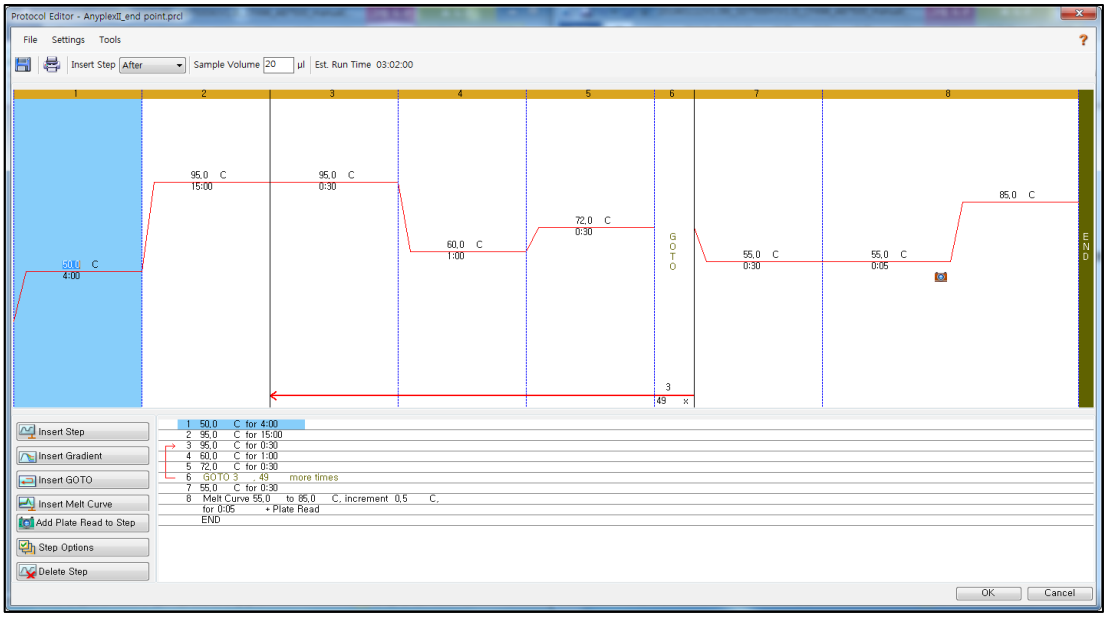


Fig. 3. **Protocol Editor** (End point-CMTA)

3) Click on **Sample Volume** to directly edit the 20 µL.

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

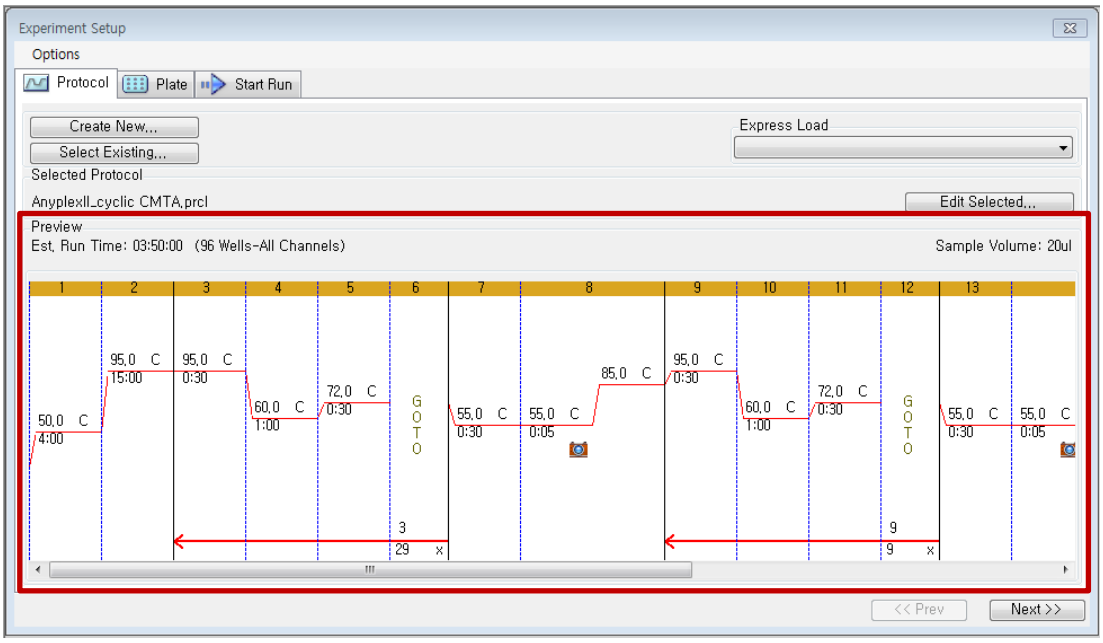


Fig. 4. **Experiment Setup Protocol** (cyclic-CMTA)

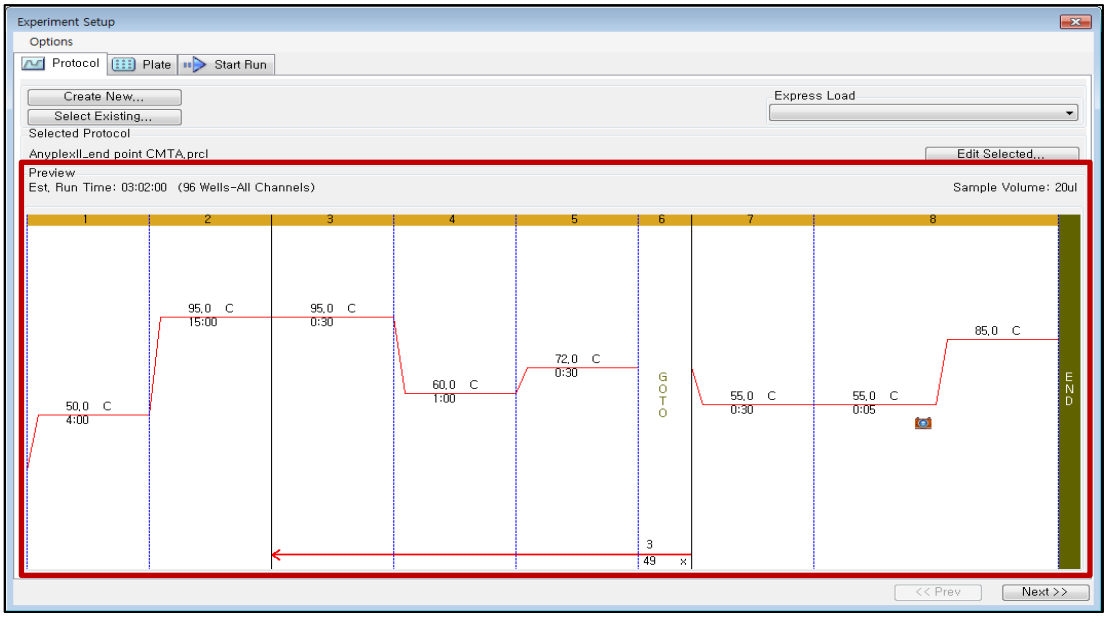


Fig. 5. **Experiment Setup Protocol** (End point-CMTA)

B. Plate Setup

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

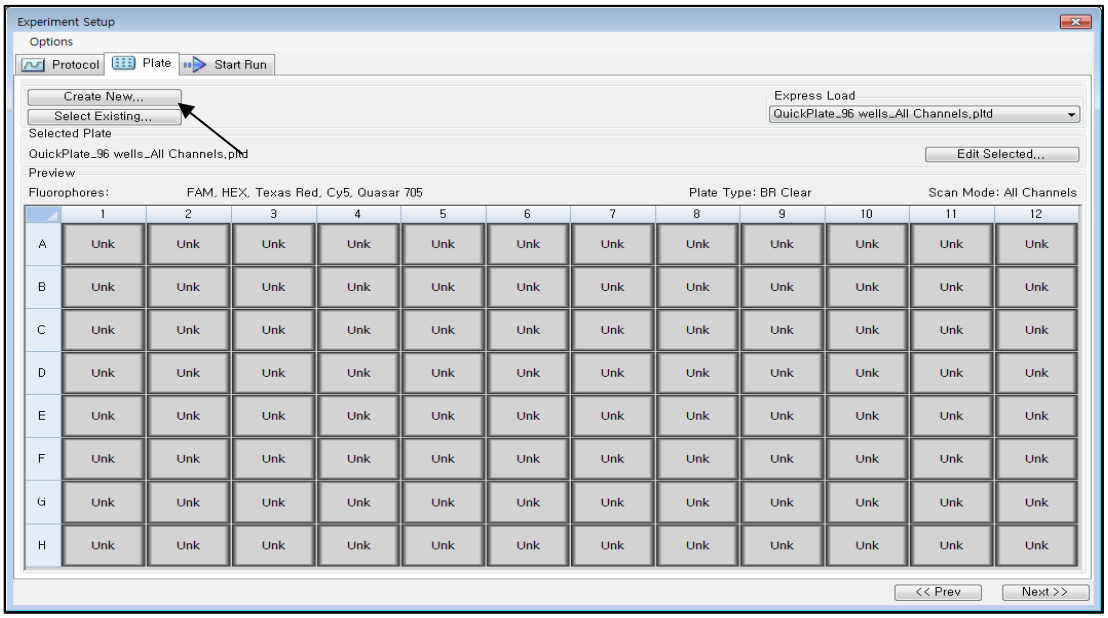


Fig. 6. **Plate Editor**

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**.

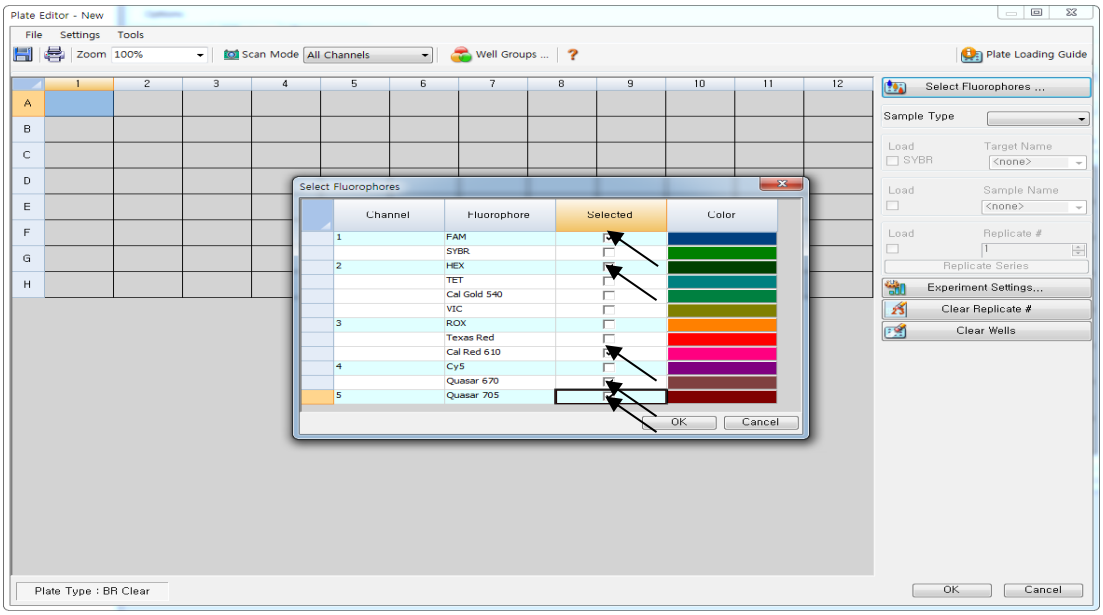


Fig. 7. **Select Fluorophores** (**FAM**, **HEX**, **Cal Red 610, Quasar 670** and **Quasar 705**)

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)**.

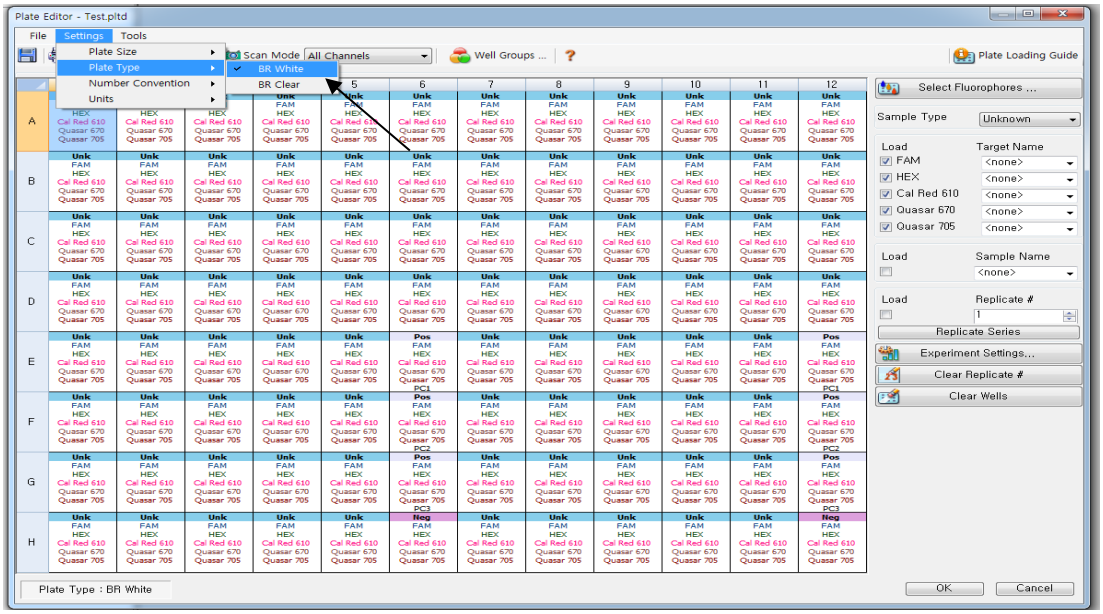


Fig. 8. **Plate Setup**

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

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

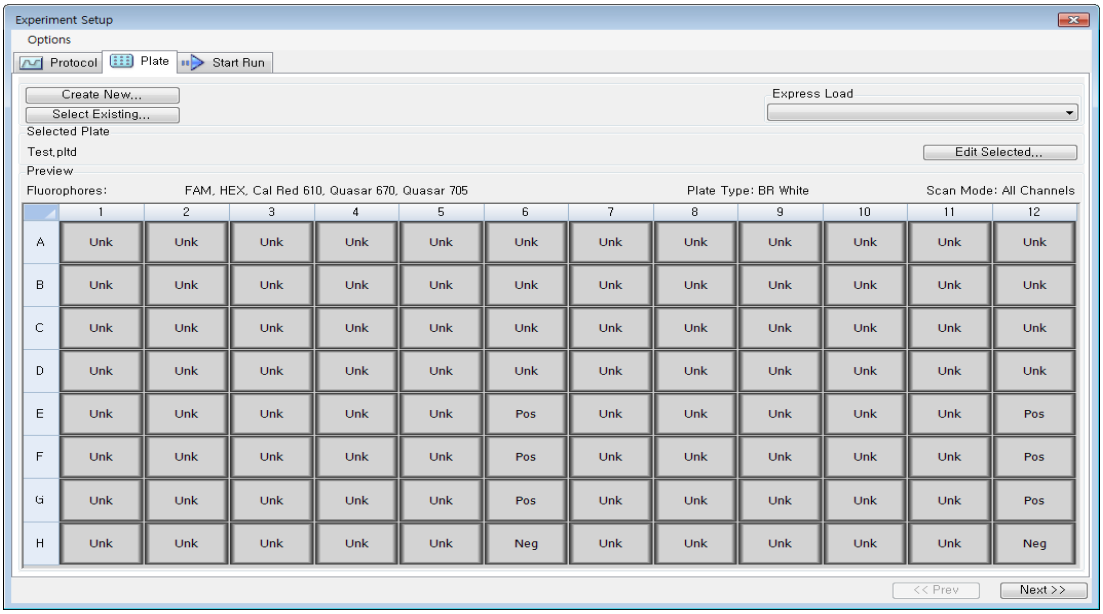


Fig. 9. **Experiment Setup: Plate**

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.

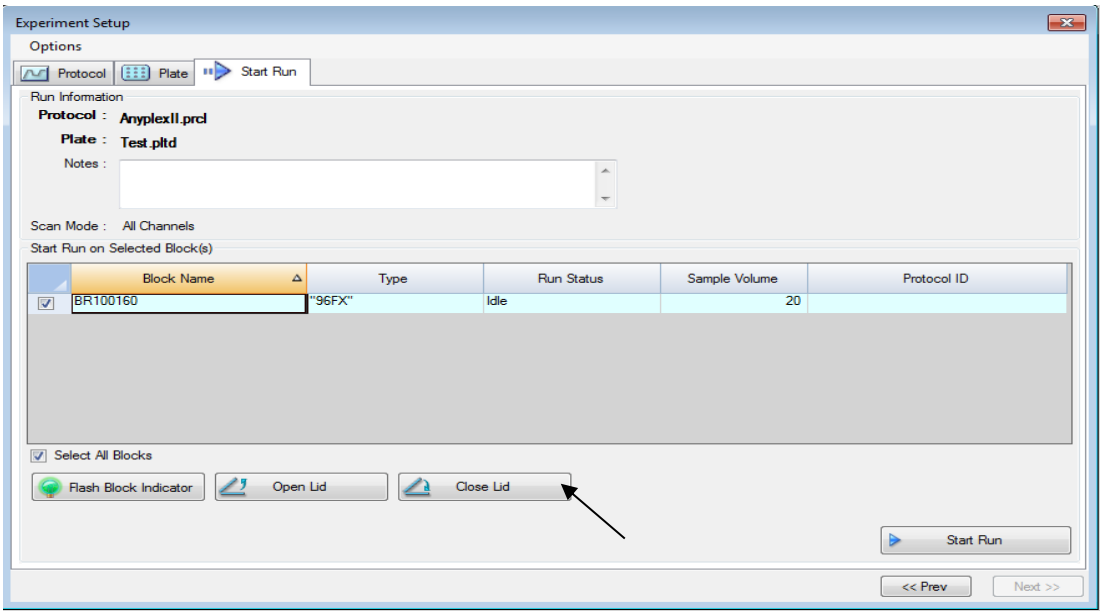


Fig. 10. **Close Lid**

2) Select the instrument checkbox and click **Start Run**.

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

1.2. Data Analysis

A. Create folders for data export

A-1. cyclic-CMTA

- **When using 'Export All Data Sheets to Excel' function (See page 32)**

1) To save data from each melt curve detection step from the results file, create three folders for each step: "1" for data from step 8, "2" for data from step 14, and "3" for data from step 20.

- **When using 'Seegene Export' function (See page 36)**

1) To save data from all of the melt curve detection steps from the results file, create one folder.

2) Folder name may be as desired by the user (For 'Seegene Export' function, MeltStep8, MeltStep14 and MeltStep20 are automatically created to save each melt point data under the folder created by the user).

A-2. End point-CMTA

1) To save data for melt point from result file, create one folder.

2) Folder name may be as desired by the user.

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

B-1. Using 'Export All Data Sheets to Excel' function

1) After the test, click the Melt curve tab to confirm the Melt Peak results.

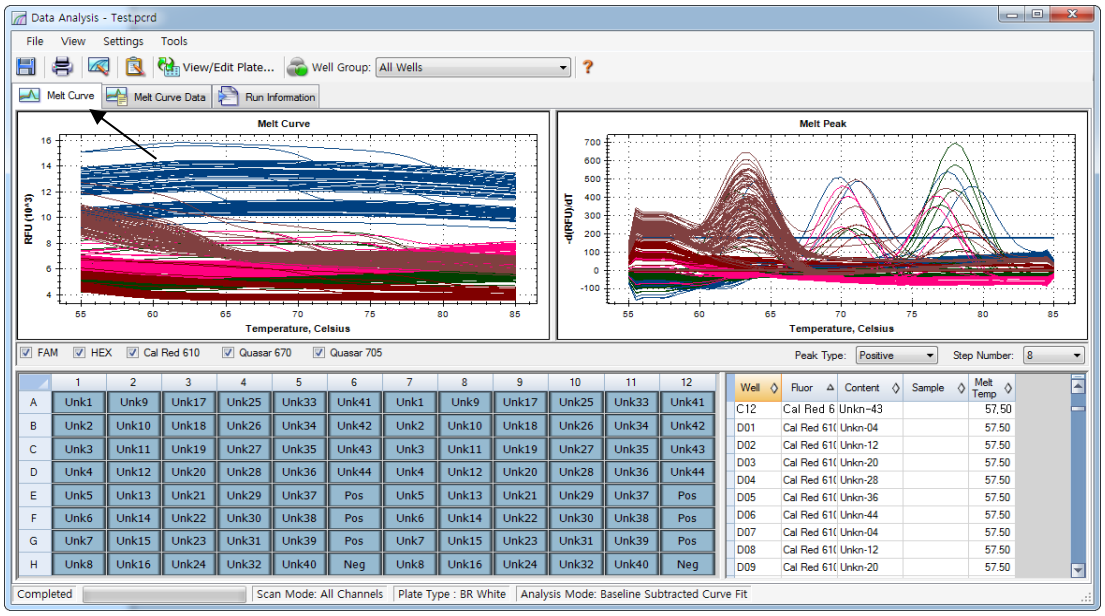


Fig. 11. **Melt Peak results**

2) Select **Step number** "**8**" and select "**Export All Data Sheets to Excel**" from Tools menu.

Note: Select "Export All Data Sheets to Excel" directly in case of End point-CMTA.

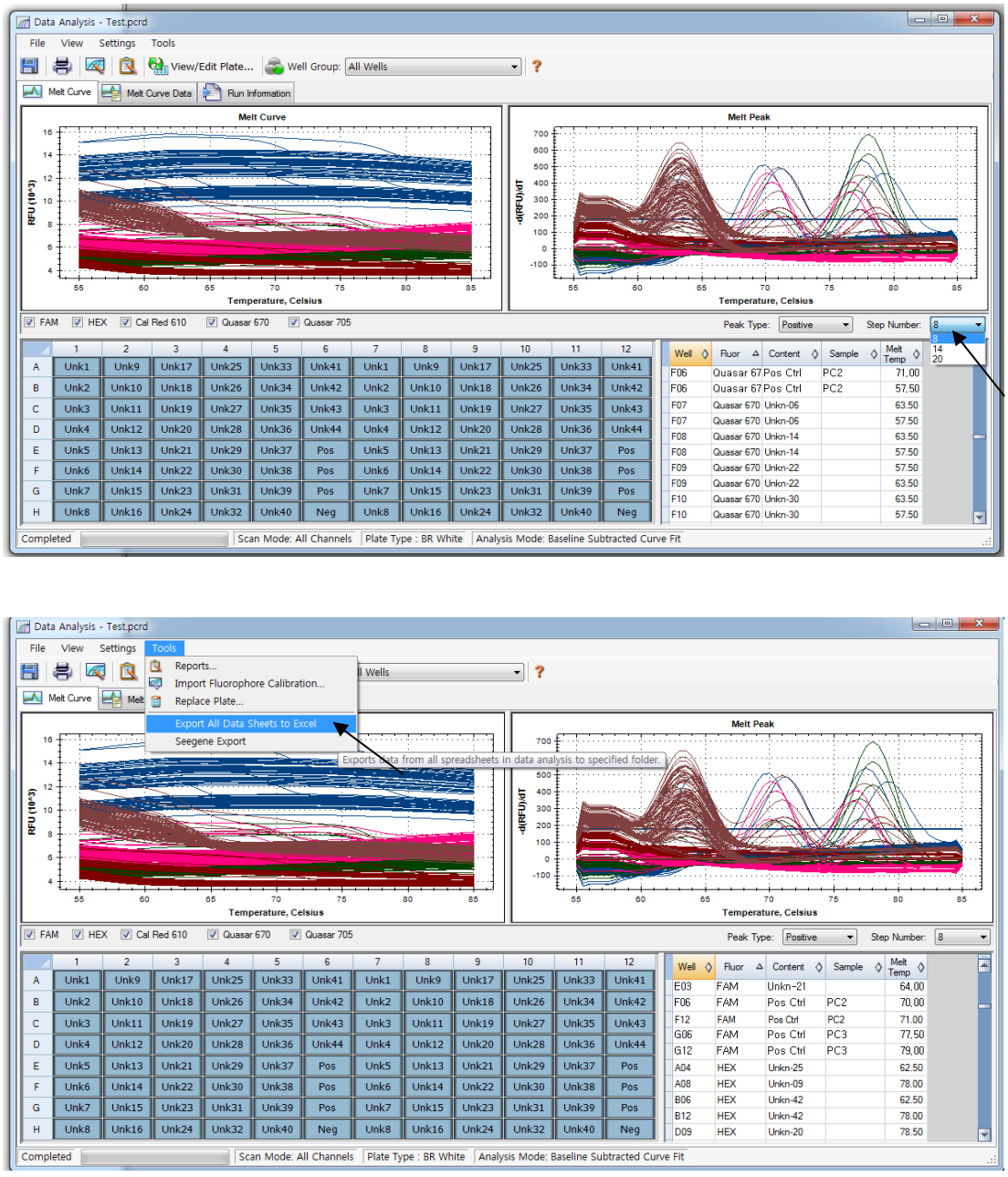


Fig. 12. **Export All Data Sheets to Excel**

3) Save the result to the specified folder “1”.

Note: In case of End point-CMTA, results can be saved in any folder.

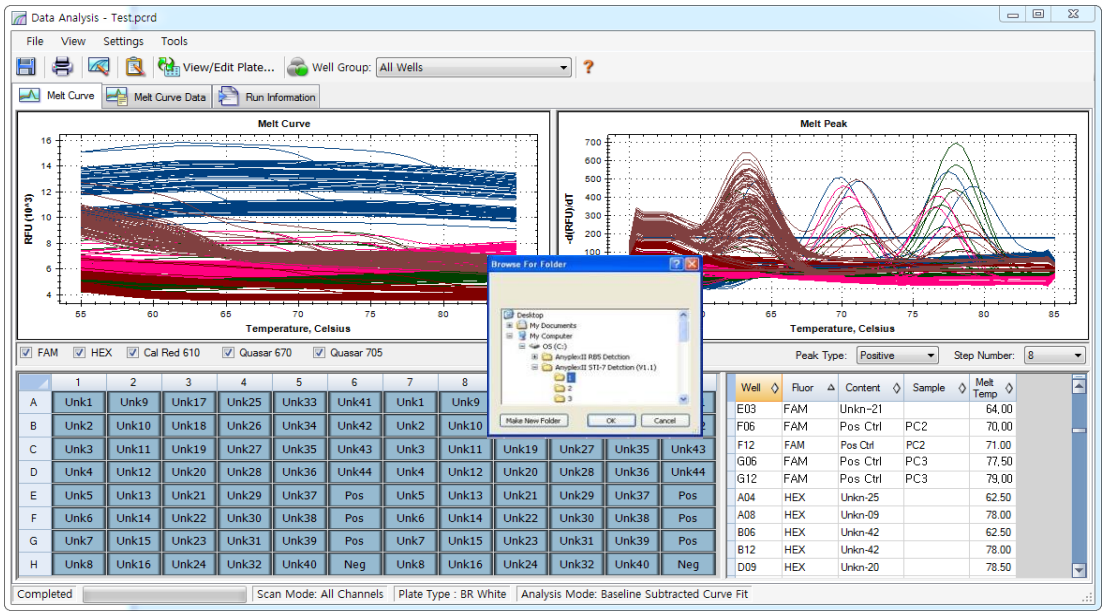


Fig. 13. **Export all data from spreadsheets to designated folder**

4) Make sure that the results have been saved to the folder “1”.

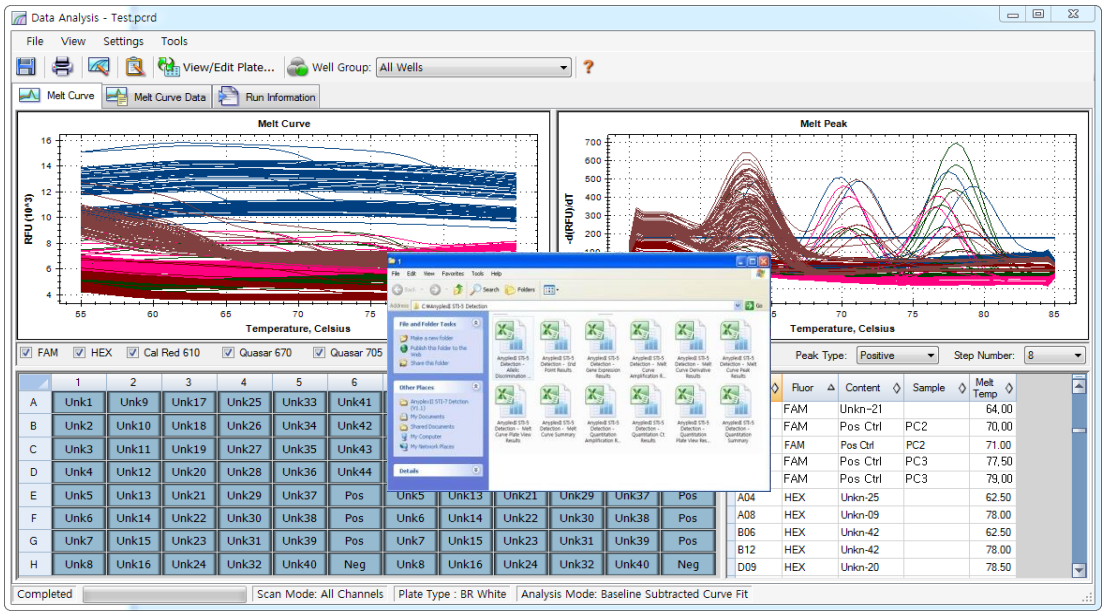


Fig. 14**. Exported Result files**

Note: Skip 5) ~ 7) steps and process next analysis stage in case of End point-CMTA.

5) Return to step 2) and select **Step number** "**14**". Repeat steps 3) & 4) and save data in designated folder **"2"**.

6) Return back to step 2) and select **Step number "20"**.

7) Repeat steps 3) & 4) and save data in **"3" folder**. Data of each step number is saved as shown below.

Step number	Designated folder
8	1
14	2
20	3

B-2. Using 'Seegene Export' function

1) After the test, click the **Melt Curve** tab to confirm the Melt Peak results.

Fig. 15. **Melt Peak results**

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

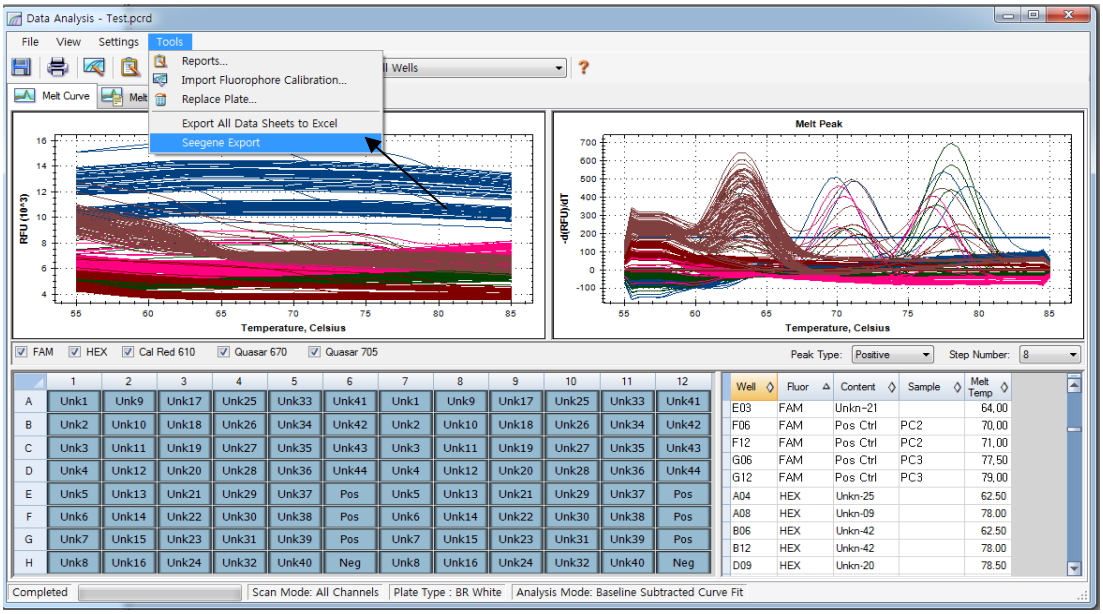


Fig. 16. **Seegene Export**

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

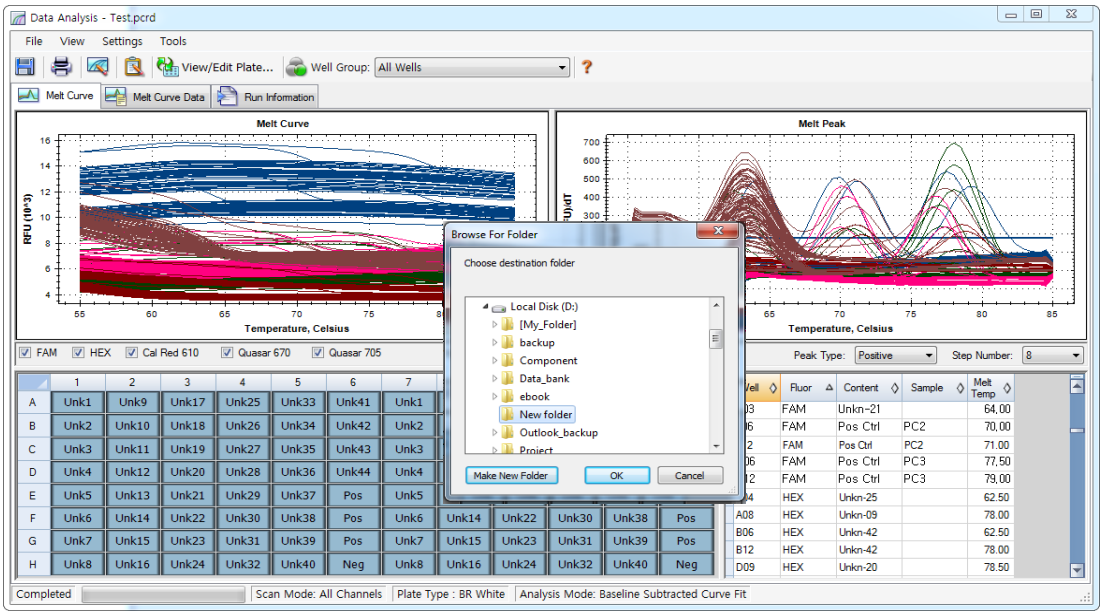


Fig. 17. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

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

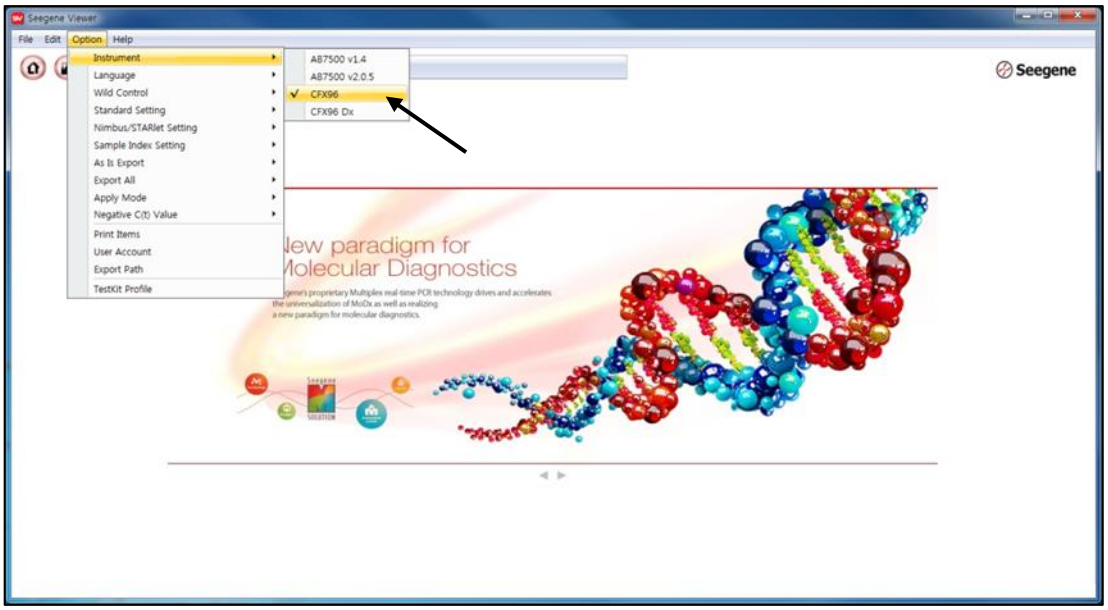


Fig. 18. **Seegene viewer**

2) Click **Open** to find the saved file in folder "1"or folder “MeltStep8”, open the results file, and select the test kit from the **PRODUCT** menu.

Note: Please find the saved data in arbitrary folder in case of End point-CMTA.

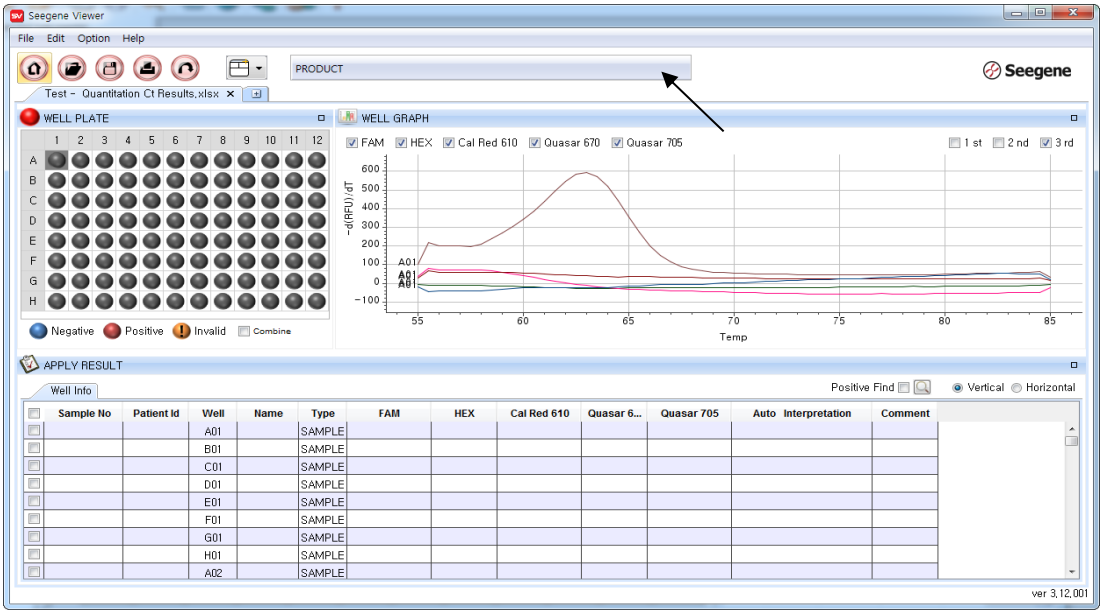


Fig. 19. **Settings for Data Analysis in Seegene Viewer**

Note: Please verify the type of tube when selecting test kit (8 strip / 96 plate / 96 film).

3) Check the result for each well.

Fig. 20. **Test result on Seegene Viewer**

2. CFX96™ Dx System (CFX Manager™ Dx Software 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**.

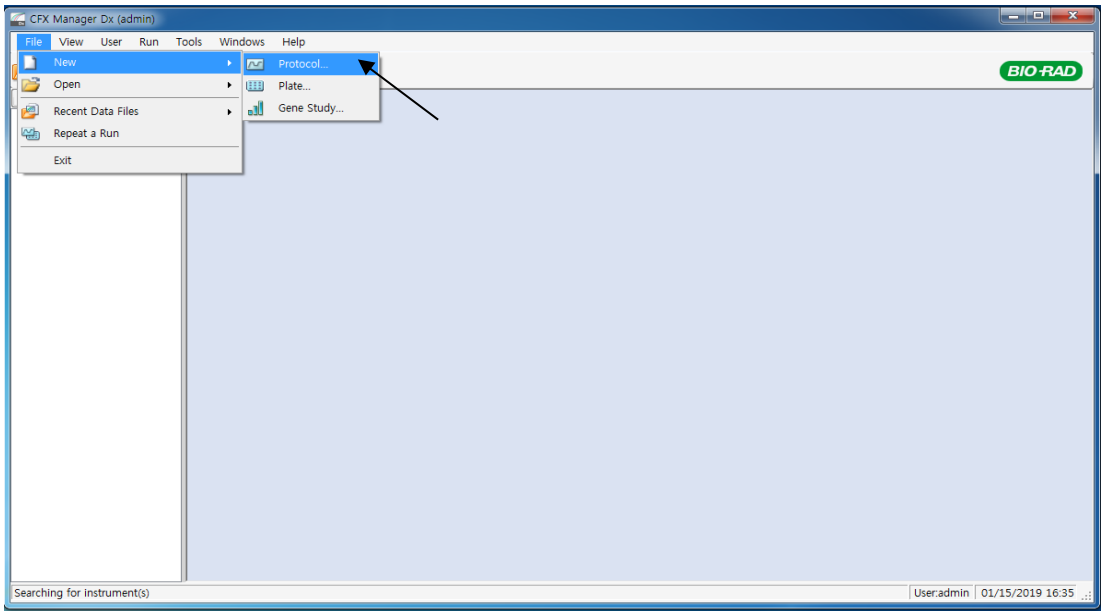


Fig. 1. **Protocol Setup**

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

i) cyclic-CMTA (Melt analysis of three times)

Step	Temperature	Duration	No. of cycles
1	50°C	4 min	
2	95°C	15 min	
3	95°C	30 sec	30
4	60°C	1 min	
5	72°C	30 sec	
6	GOTO 3, 29 more times		
7	55°C	30 sec	
8*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		
9	95°C	30 sec	10
10	60°C	1 min	
11	72°C	30 sec	
12	GOTO 9, 9 more times		
13	55°C	30 sec	
14*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		
15	95°C	30 sec	10
16	60°C	1 min	
17	72°C	30 sec	
18	GOTO 15, 9 more times		
19	55°C	30 sec	
20*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		

***Note: Plate Read at Steps 8, 14 and 20.** Fluorescence is detected at Melting.

ii) End point-CMTA (Melt analysis of one time)

Step	Temperature	Duration	No. of cycles
1	50°C	4 min	
2	95°C	15 min	
3	95°C	30 sec	50
4	60°C	1 min	
5	72°C	30 sec	
6	GOTO 3, 49 more times		
7	55°C	30 sec	
8*	Melting curve 55°C ~ 85°C (5 s / 0.5°C)		

***Note: Plate Read at Step 8.** Fluorescence is detected at Melting**.**

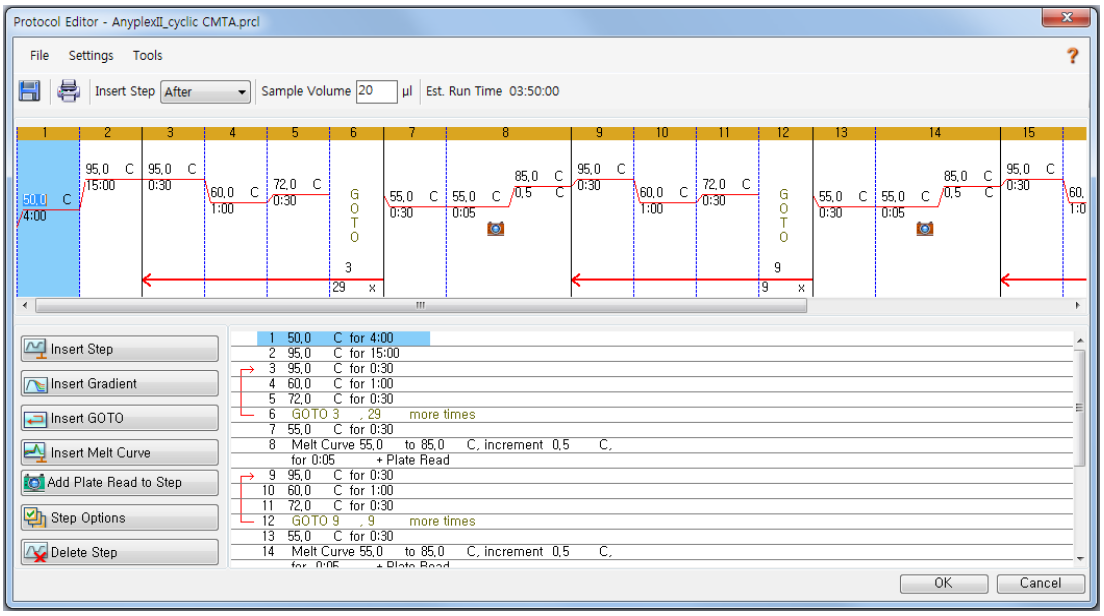


Fig. 2. **Protocol Editor** (cyclic-CMTA)

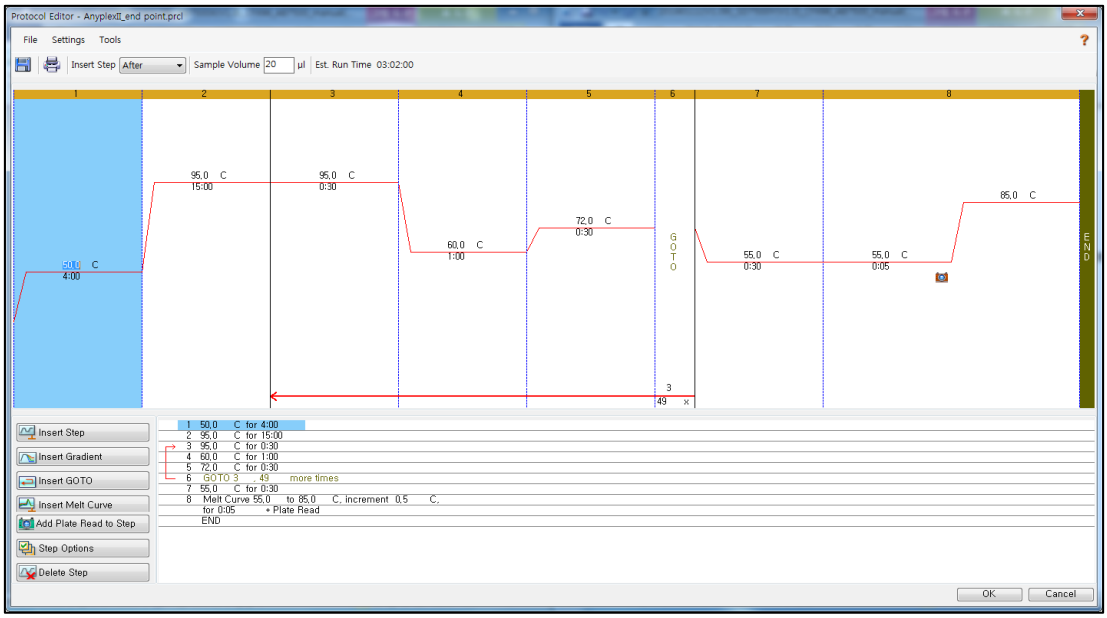


Fig. 3. **Protocol Editor** (End point-CMTA)

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.

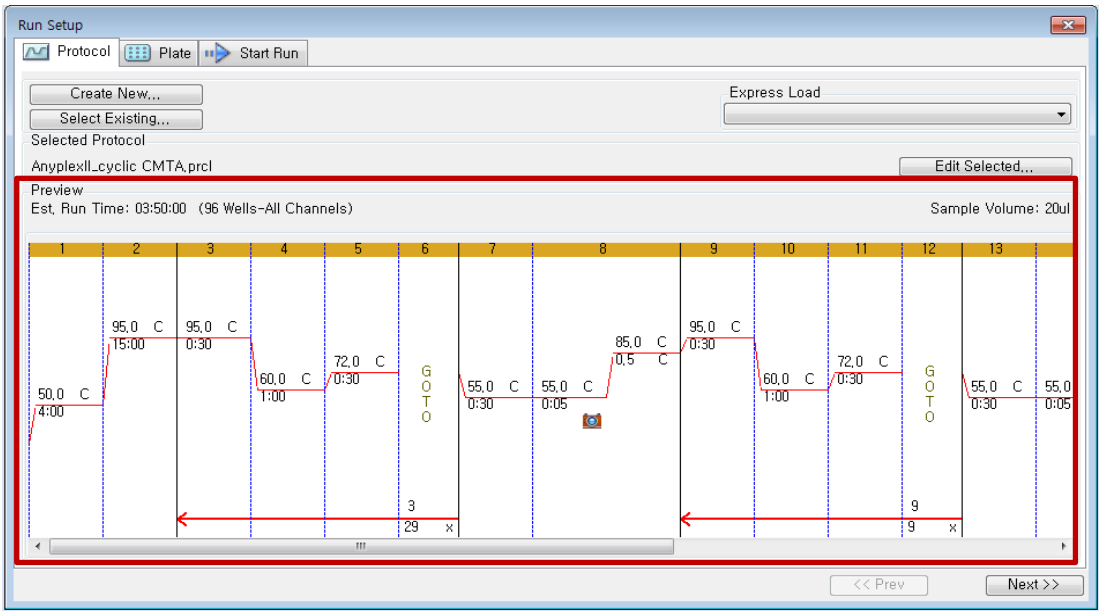


Fig. 4. **Run Setup** Protocol (cyclic-CMTA)

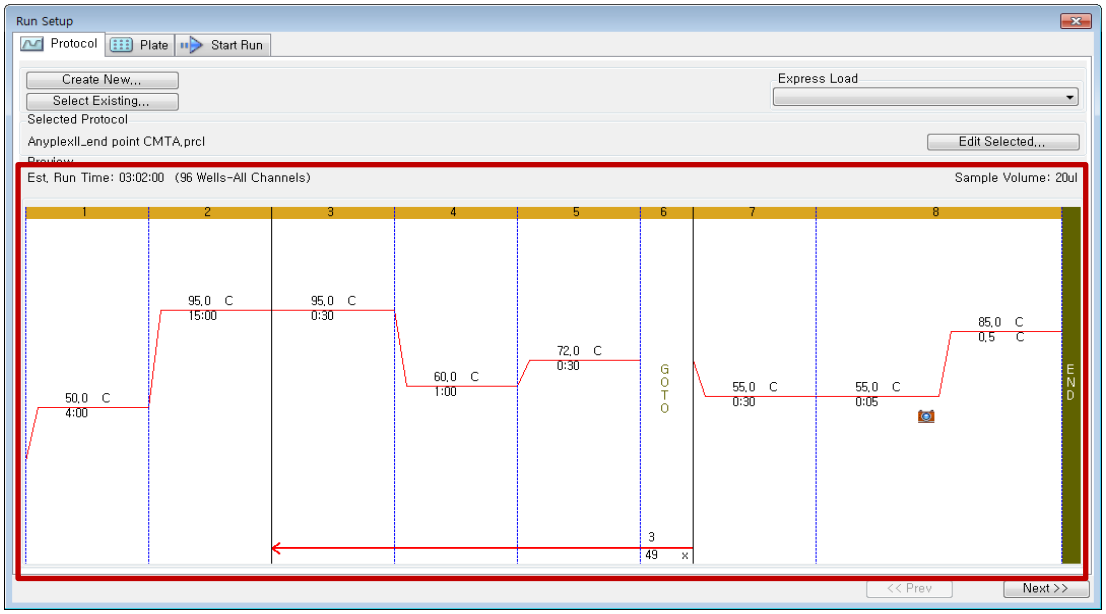


Fig. 5. **Run Setup Protocol** (End point-CMTA)

B. Plate Setup

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

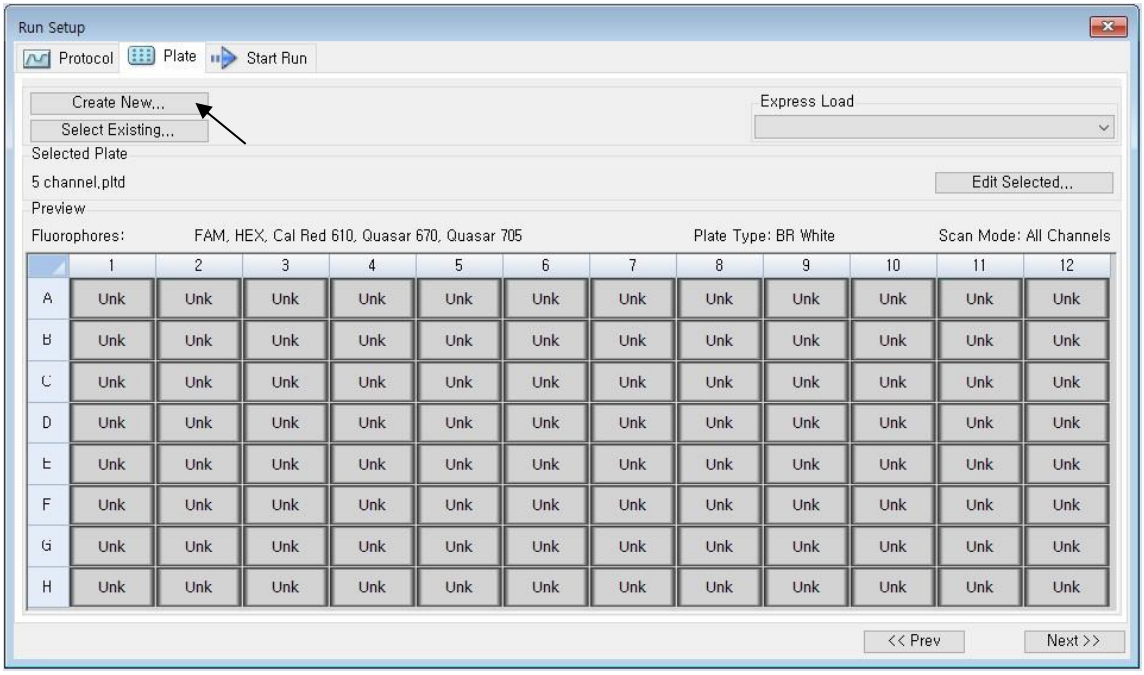


Fig. 6. **Plate Editor**

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**.

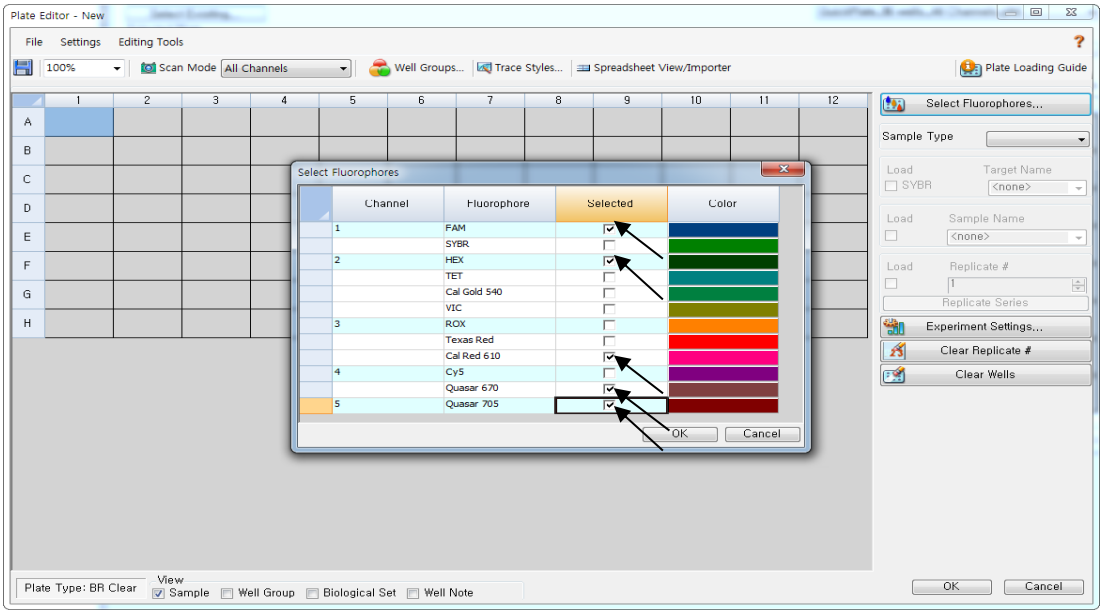


Fig. 7. **Select Fluorophores** (**FAM**, **HEX**, **Cal Red 610**, **Quasar 670 and Quasar 705**)

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)**.

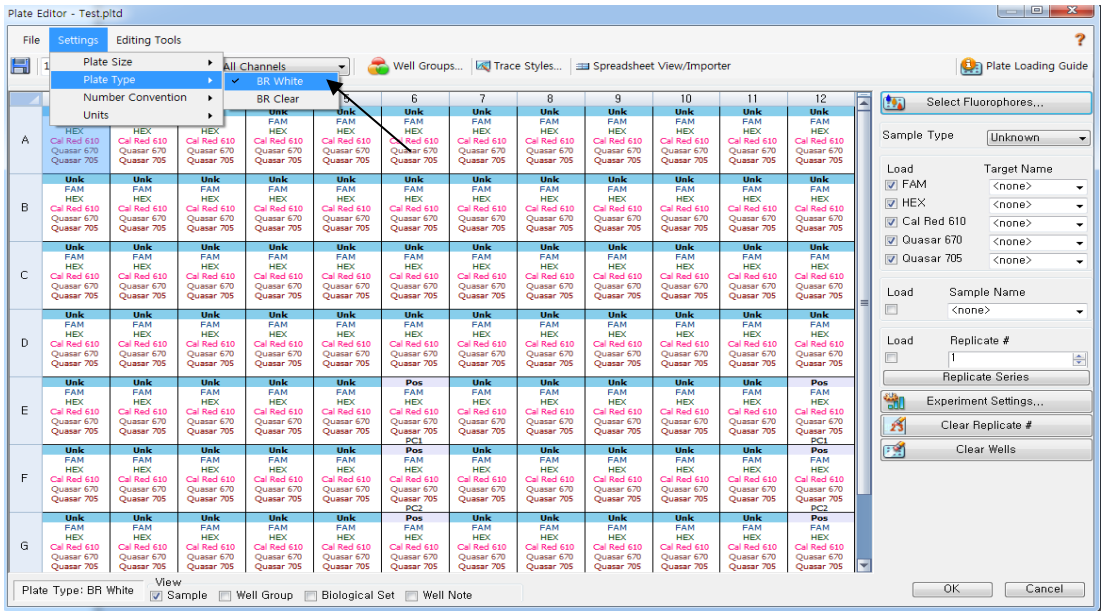


Fig. 8. **Plate Setup**

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

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

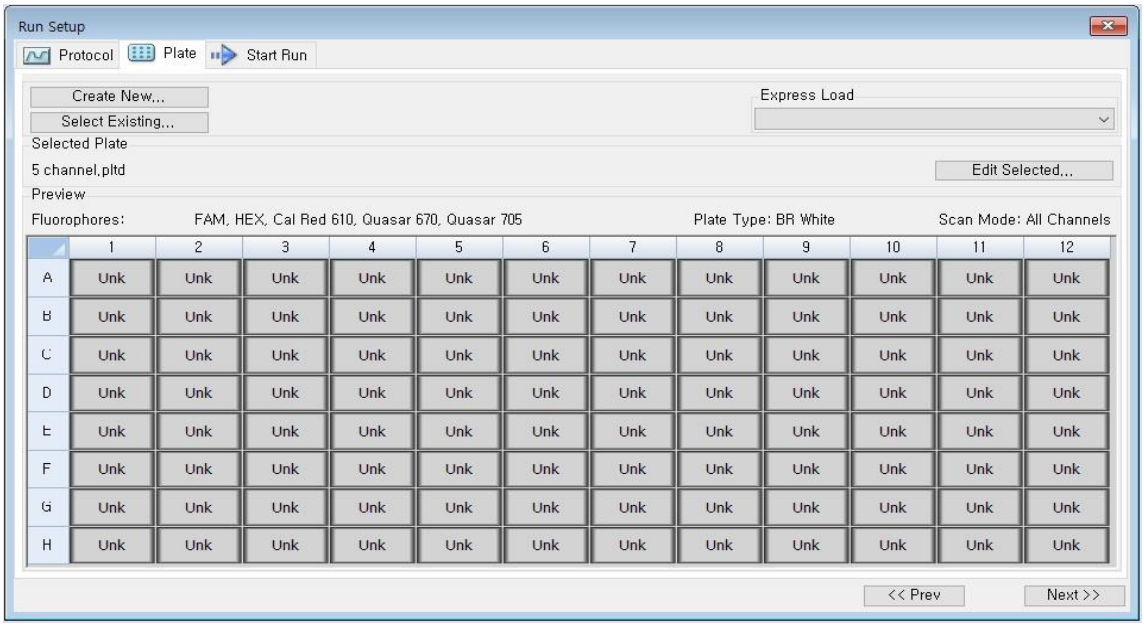


Fig. 9. **Run Setup: Plate**

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.

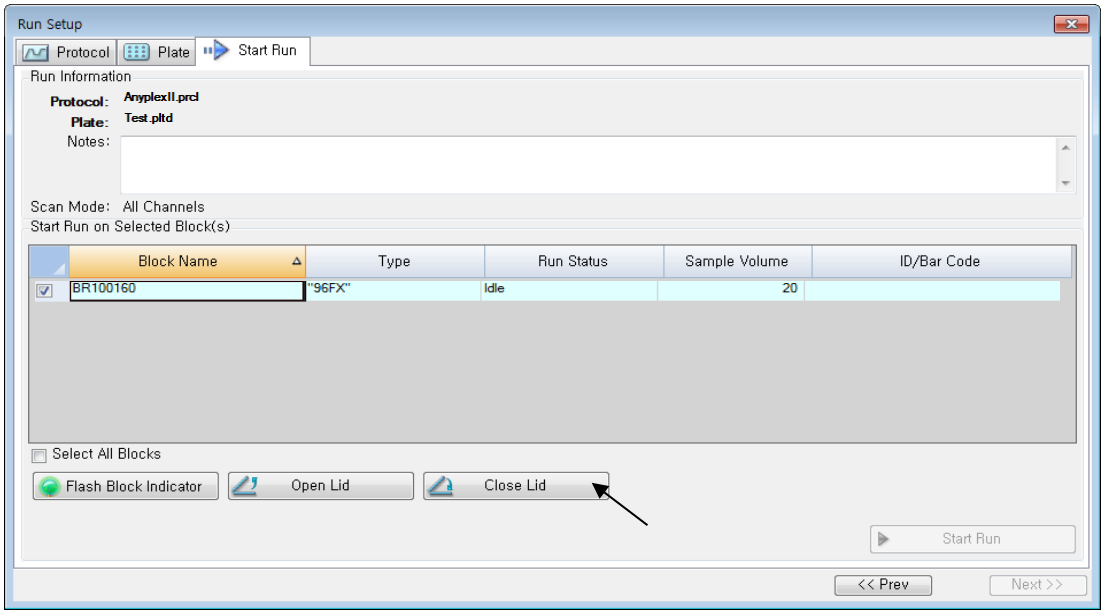


Fig. 10. **Close Lid**

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

A-1. cyclic-CMTA

• When using 'Export All Data Sheets to Excel' function (See page 48)

1) To save data from each melt curve detection step from the results file, create three folders for each step: "1" for data from step 8, "2" for data from step 14, and "3" for data from step 20.

• When using 'Seegene Export' function (See page 52)

1) To save data from all of the melt curve detection steps from the results file, create one folder.

2) Folder name may be as desired by the user (For 'Seegene Export' function, MeltStep8, MeltStep14, and MeltStep20 are automatically created to save each melt point data under the folder created by the user).

A-2. End point-CMTA

1) To save data for melt point from result file, create one folder.

2) Folder name may be as desired by the user.

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

B-1. Using 'Export All Data Sheets to Excel' function

1) After the test, click the **Melt Curve** tab to confirm the Melt Peak results.

Fig. 11. **Melt Peak results**

2) Select **Step Number** "**8**" and select **"Export All Data Sheets (Excel 2007 or Excel 2003)**" from Export menu.

Note: Select "Export All Data Sheets (Excel 2007 or Excel 2003)" directly in case of End point-CMTA.

Fig. 12**. Export All Data Sheets to Excel**

3) Save the result to the specified folder “1”.

Note: In case of End point-CMTA, results can be saved in any folder.

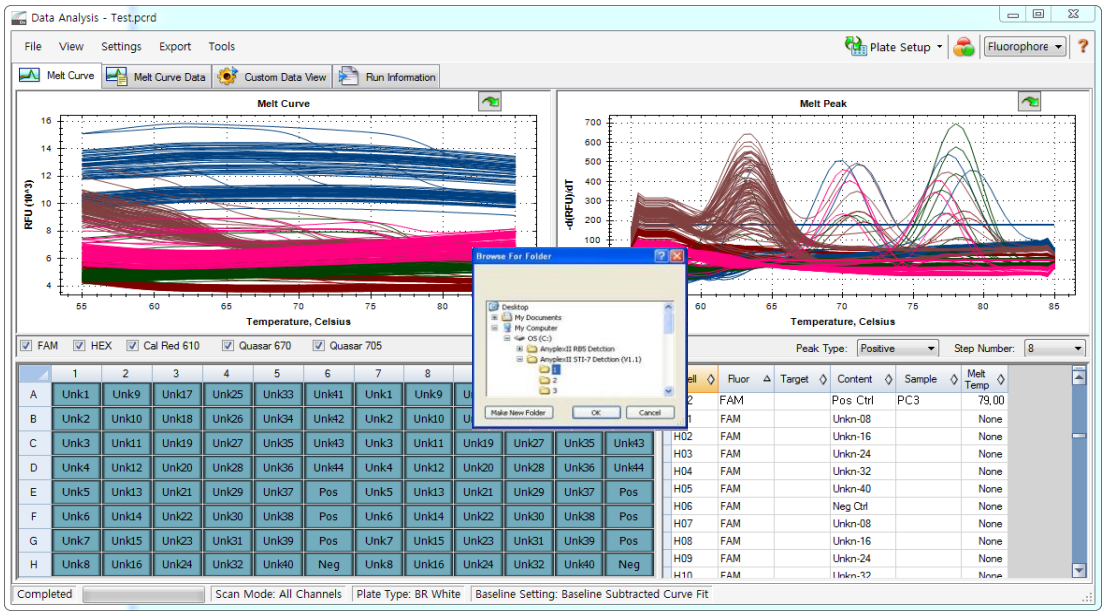


Fig. 13. **Export all data from spreadsheets to designated folder**

4) Make sure that the results have been saved to the folder “1”.

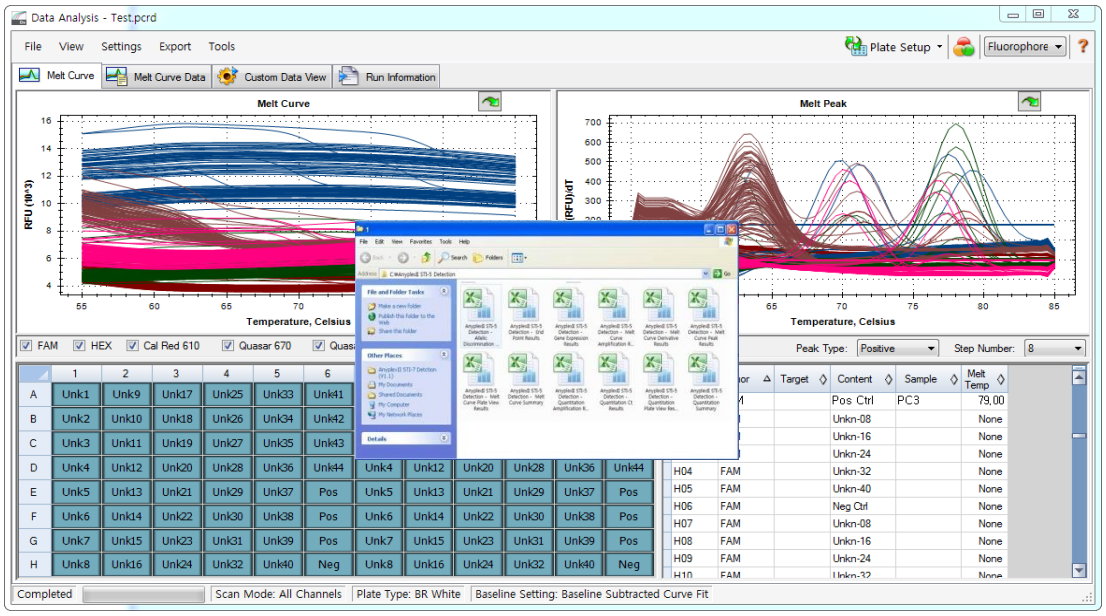


Fig. 14**. Exported Result files**

Note: Skip 5) ~ 7) steps and process next analysis stage in case of End point-CMTA.

5) Return to step 2) and select **Step number** "**14**". Repeat steps 3) & 4) and save data in designated folder **"2"**.

6) Return back to step 2) and select **Step number "20"**.

7) Repeat steps 3) & 4) and save data in **"3" folder**. Data of each step number is saved as shown below.

Step number	Designated folder
8	1
14	2
20	3

B-2. Using 'Seegene Export' function

1) After the test, click the **Melt Curve** tab to confirm the Melt Peak results.

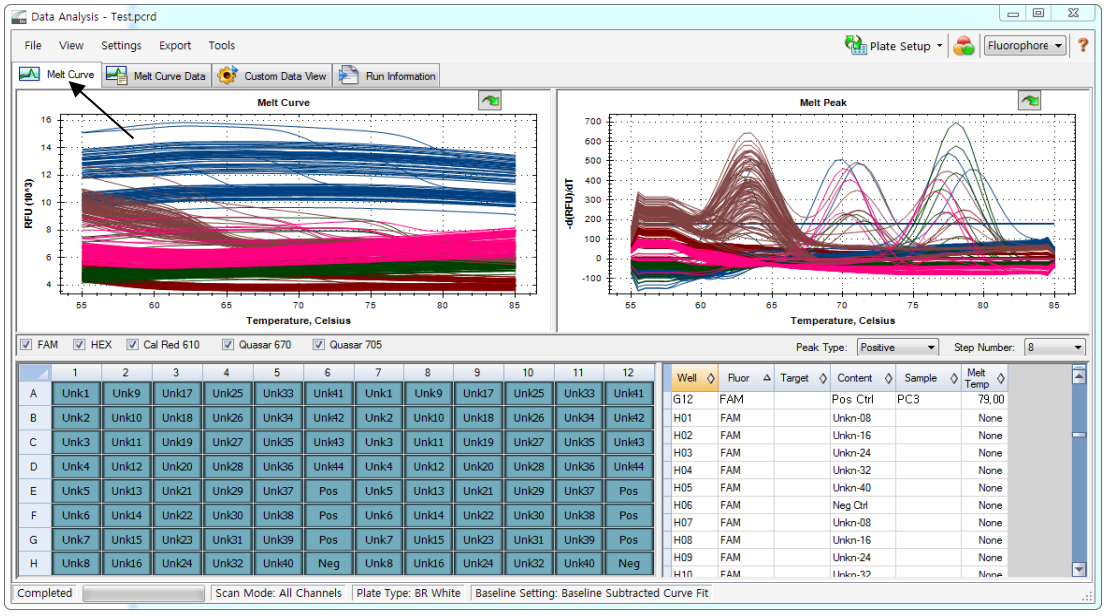


Fig. 15**. Melt Peak results**

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

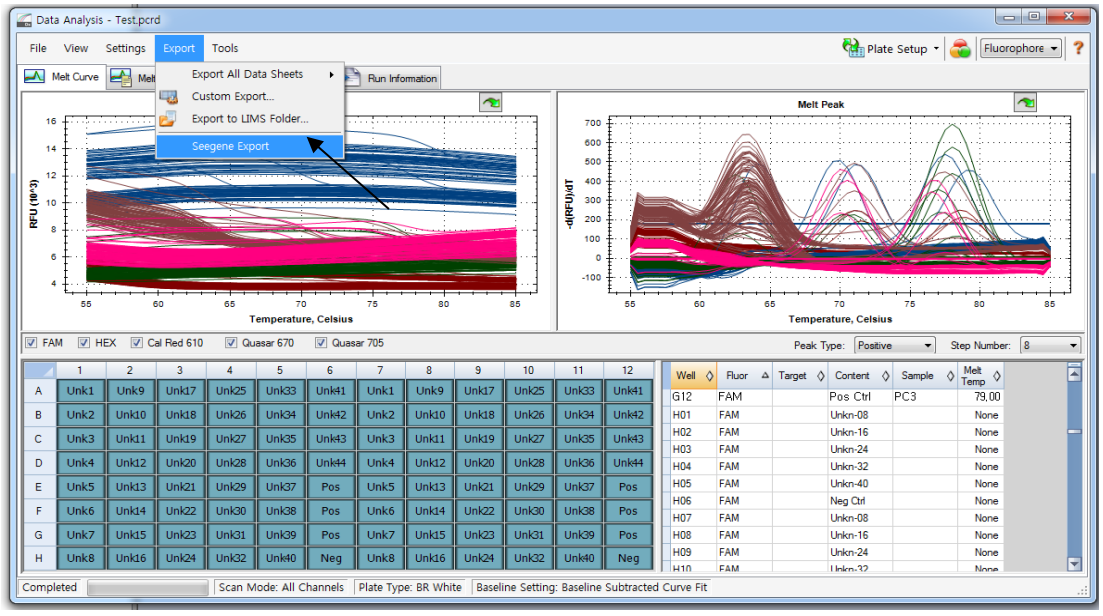


Fig. 16**. Seegene Export**

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

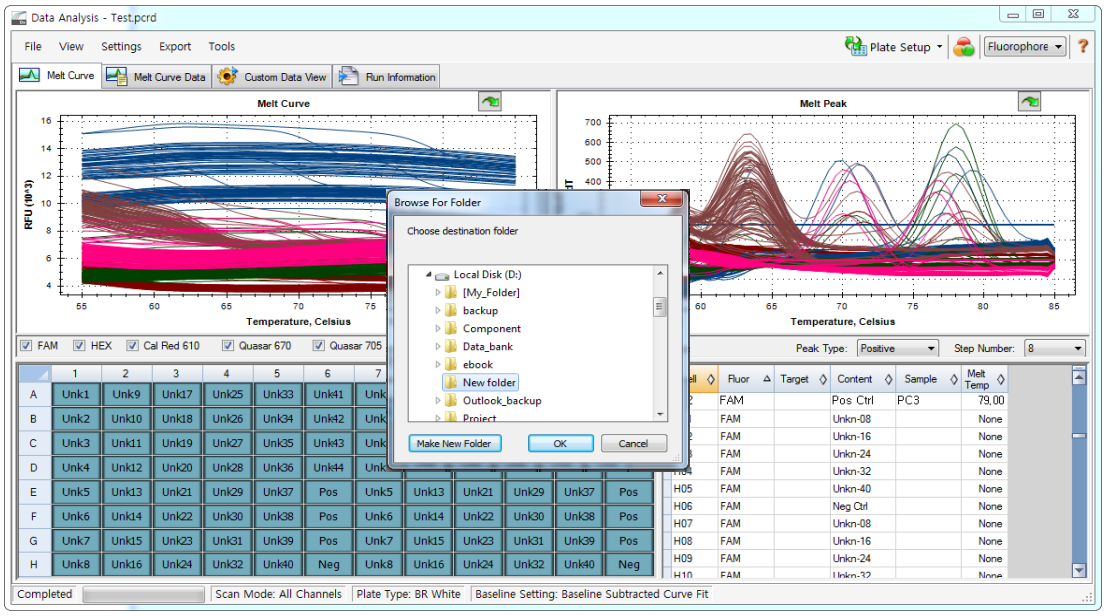


Fig. 17**. Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

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

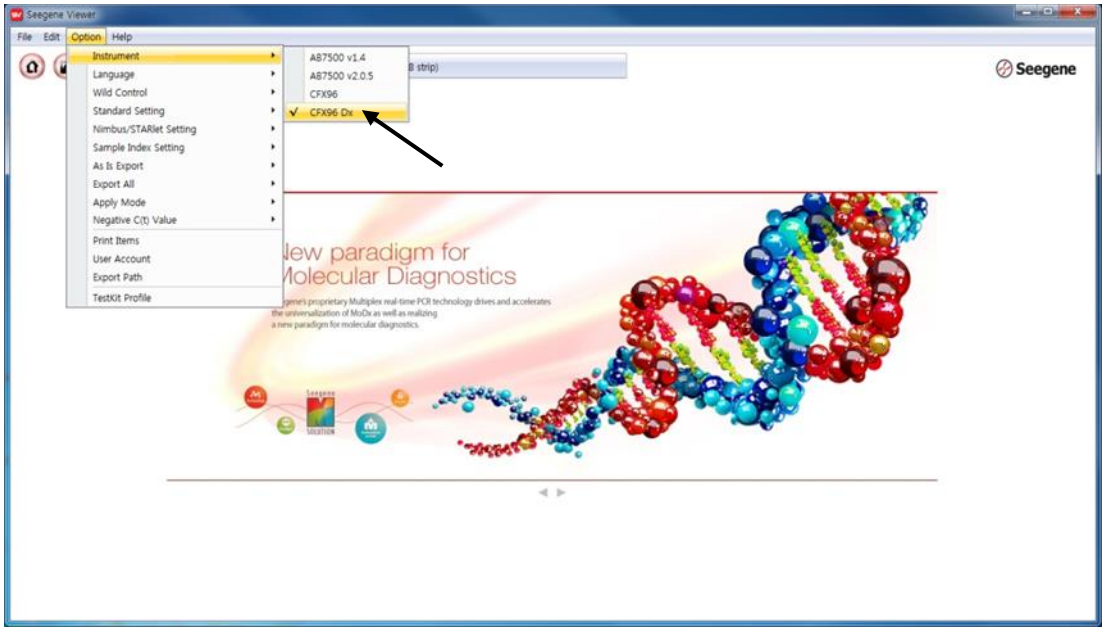


Fig. 18. **Seegene viewer**

2) Click **Open** to find the saved file in folder "1"or folder “MeltStep8”, open the results file, and select the test kit from the **PRODUCT** menu.

Note: Please find the saved data in arbitrary folder in case of End point-CMTA.

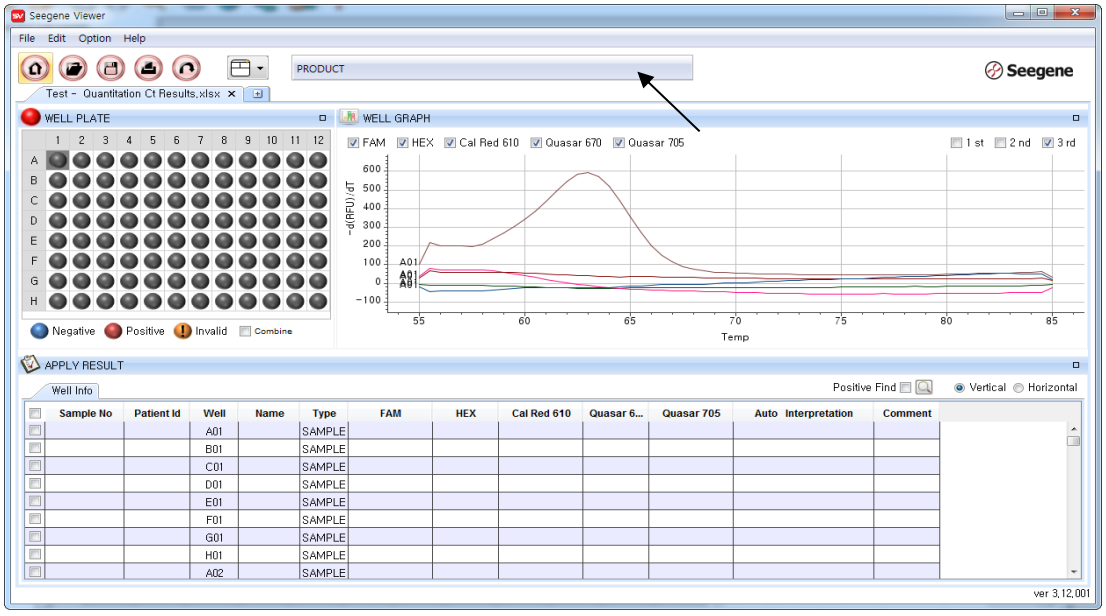


Fig. 19. **Settings for Data Analysis in Seegene Viewer**

Note: Please verify the type of tube when selecting test kit (8 strip / 96 plate / 96 film).

3) Check the result for each well.

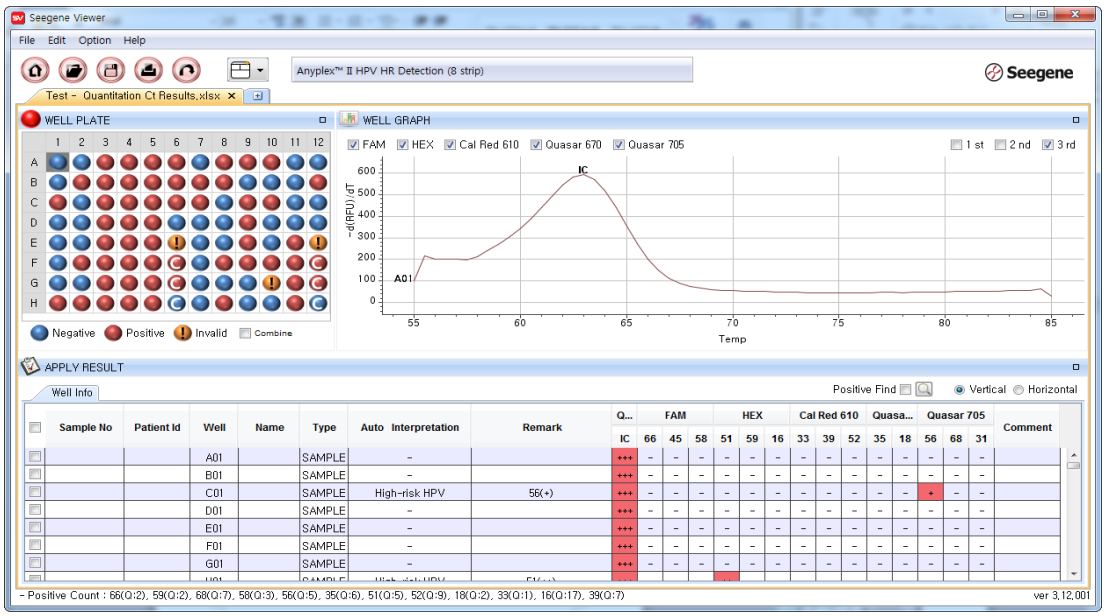
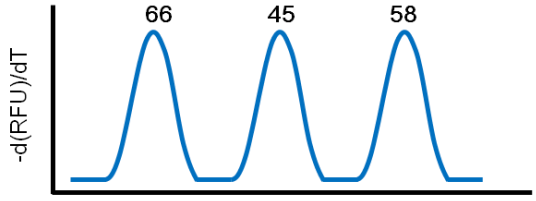
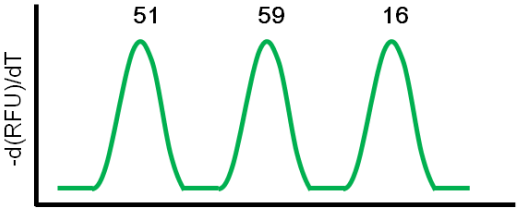
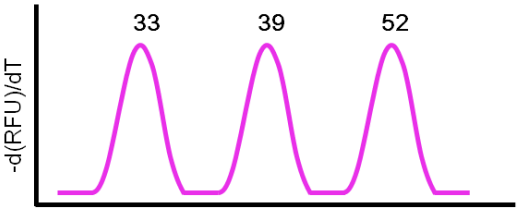
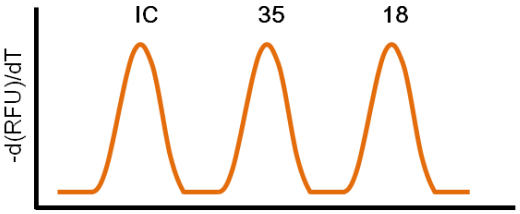
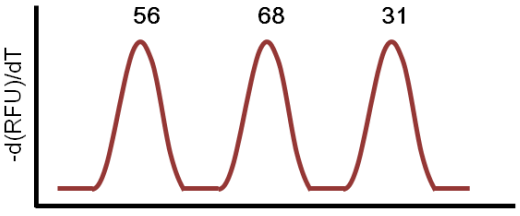


Fig. 20. **Test result on Seegene Viewer**

RESULTS

1. Analyte Information

Fluorophore	Anyplex™ II HPV HR Detection
FAM	
HEX	
Cal Red610	
Quasar670	
Quasar705	

2. Interpretation of Results

A. cyclic-CMTA

HPV Result*	IC Result*	Interpretation
+++ or ++ or +	+++ or ++	**Target Nucleic acid, detected** - Target HPV type identification
+++ or ++ or +	+ or -	**Target Nucleic acid, detected**** - Target HPV type identification - Additional HPV genotypes which may be present were not detected
-	+++ or ++	**Target Nucleic acid, not detected**
-	+ or -	**Invalid** - Weak or 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 (Page 60).

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

Result	cyclic-CMTA (cyclic-Catcher Melting Temperature Analysis)		
	First CMTA point	Second CMTA point	Third CMTA point
+++	+	+	+
++	-	+	+
+	-	-	+
-	-	-	-

B. End point-CMTA

HPV Result*	IC Result*	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.

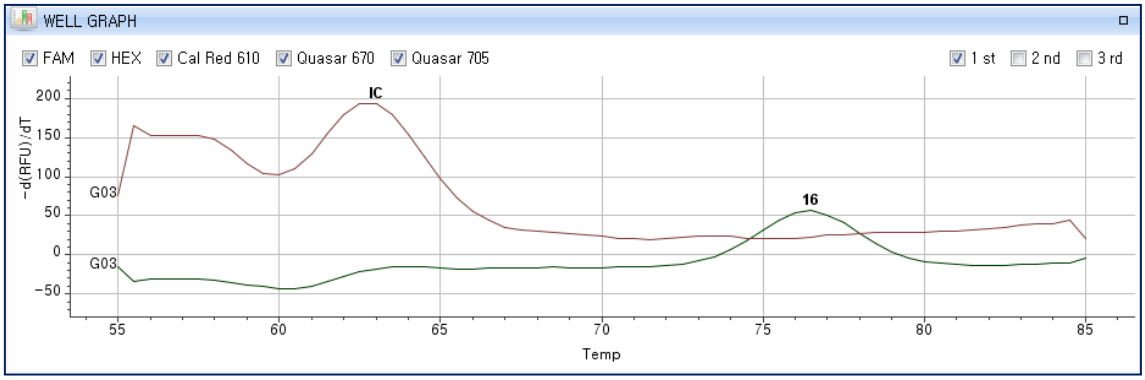
* Internal Control or any other signals are not observed: see TROUBLESHOOTINGS (Page 60).

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

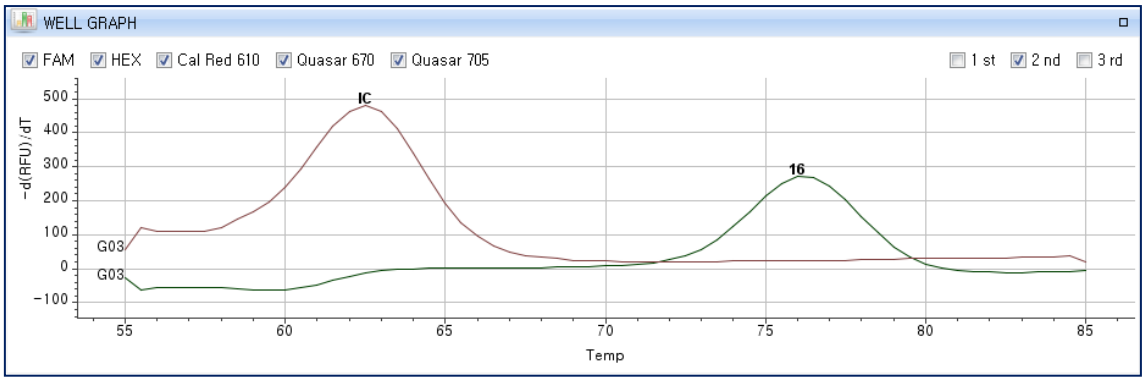
3. Application to Clinical Samples

A. cyclic-CMTA

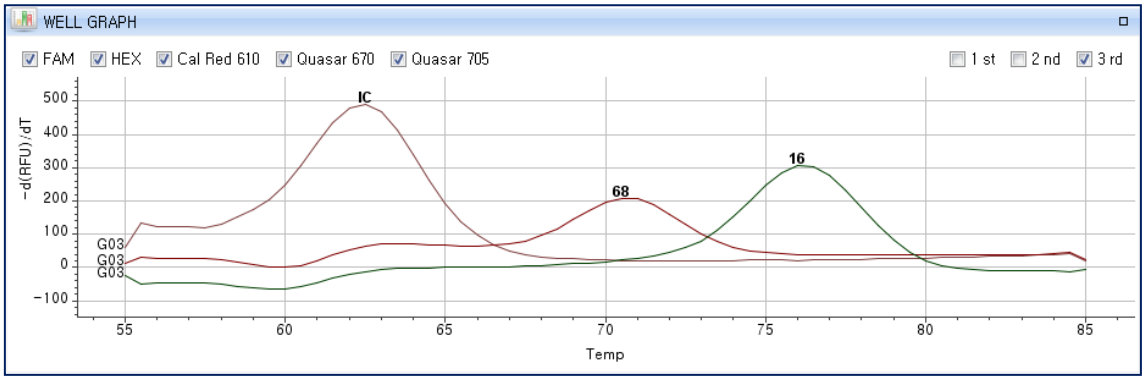
Melt Peak-1st (First CMTA point)



Melt Peak-2nd (Second CMTA point)

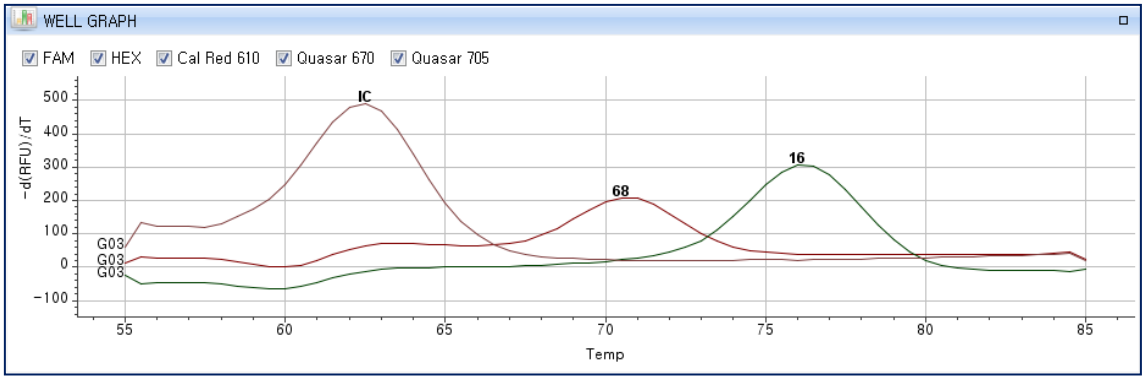


Melt Peak-3rd (Third CMTA point)



Auto Interpretation	Remark	Quasar 670	FAM			HEX			Cal Red 610			Quasar 670		Quasar 705		
High-risk HPV	16(+++), 68(+)	IC	66	45	58	51	59	16	33	39	52	35	18	56	68	31
		+++	-	-	-	-	-	+++	-	-	-	-	-	-	+	-

B. End point-CMTA



Auto Interpretation	Remark	Quasar 670	FAM			HEX			Cal Red 610			Quasar 670		Quasar 705		
High-risk HPV	16, 68	IC	66	45	58	51	59	16	33	39	52	35	18	56	68	31
		+	-	-	-	-	-	+	-	-	-	-	-	-	+	-

TROUBLESHOOTINGS

Anyplex™ II 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 PCR cycle or machine temperature	Please check the PCR conditions and repeat the PCR under the correct setting if necessary.
	Leaving reagents at room temperature for a long time or incorrect storage condition	Please check the storage conditions (See page 11) and the expiry date (see the kit label) of the reagents and use a new kit if necessary.
	Incorrect programming	Repeat the detection procedure with a correct setting.
	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	High load of pathogen's nucleic acid	Without detection of IC signal, target signal is considered as “detected” when target is observed. For IC signal detection, re-test by diluting samples. ① Dilute the template nucleic acid in RNase-free water to 10X-100X and repeat PCR. ② Dilute the specimen in PBS to 10X-100X and repeat from extraction.
	Presence of PCR Inhibitor	
Putative false positive or target signals observed in Negative Control	Presence of cross contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Use only filter tips during the extraction procedure. Change tips among tubes. Repeat the nucleic acid extraction with the new set of reagents.
	Cross-contamination between PC1, 2 and 3	Restart from extraction step or real-time PCR step.

Anyplex™ II HPV HR Detection		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative false negative or No signals observed in Positive Control	Error in specimen collection	Recollect the specimen.
	Incorrect storage of the specimen	Recollect the specimen and repeat the whole process. Make sure the product is stored in recommended conditions.
	Error in nucleic acid extraction	Re-extract the nucleic acid.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers for nucleic acid containing tubes and make sure to add nucleic acid into correct PCR tubes during detection process.
	Presence of inhibitor	Dilute the specimen in PBS (10~100x) and repeat from extraction step with the diluted specimen.
	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	Check whether all components are added or not (Sensitivity is compromised when precomposed premix is used). All reagents must be homogenized and spun 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.

PERFORMANCE

1. Specificity

The high specificity of the Anyplex™ II HPV HR Detection is ensured by the primers specifically designed for the targets in interest and the reaction condition. Anyplex™ II HPV HR Detection has been tested for cross-reactivity in 80 different pathogens: result illustrated PCR amplifications in targets only.

Organism	Strain No.	Test Result†
Acinetobacter baumannii	ATCC 15150	Not detected
Bacteroides fragilis	ATCC 25285D	Not detected
Chlamydia trachomatis	ATCC VR-577	Not detected
Corynebacterium genitalium	ATCC 33030	Not detected
Enterobacter cloacae	KCTC 13047	Not detected
Enterococcus faecalis	ATCC 700802D-5	Not detected
Escherichia coli	ATCC 15489	Not detected
Fusobacterium nucleatum	ATCC 25586D-5	Not detected
Gardnerella vaginalis	ATCC 14019	Not detected
Haemophilus ducreyi	ATCC 33940	Not detected
Klebsiella pneumoniae	ATCC 13883	Not detected
Lactobacillus acidophilus	ATCC 4357D-5	Not detected
Lactobacillus crispatus	ATCC 33820	Not detected
Lactobacillus gasseri	ATCC 33323	Not detected
Lactobacillus iners	ATCC 55195	Not detected
Lactobacillus jensenii	ATCC 25258	Not detected
Mobiluncus curtisii	ATCC 35241	Not detected
Mobiluncus mulieris	ATCC 35243	Not detected
Neisseria gonorrhoeae	ATCC 700825D	Not detected
Neisseria meningitidis	ATCC 700532D	Not detected
Neisseria sicca	ATCC 29256	Not detected
Peptostreptococcus anaerobius	ATCC 49031D-5	Not detected
Propionibacterium acnes	ATCC 6919	Not detected
Proteus mirabilis	ATCC 12453	Not detected

Organism	Strain No.	Test Result†
Proteus vulgaris	ATCC 6059	Not detected
Pseudomonas aeruginosa	ATCC 15522	Not detected
Pseudomonas fluorescens	KCTC 49642	Not detected
Serratia marcescens	ATCC 27137D-5	Not detected
Staphylococcus aureus subsp.aureus	ATCC 29213	Not detected
Streptococcus agalactiae	ATCC BAA-611D	Not detected
Streptococcus mitis	ATCC 49456D-5	Not detected
Streptococcus pyogenes	ATCC 700294D-5	Not detected
Trichomonas vaginalis	ATCC 30001D	Not detected
Ureaplasma urealyticum	ATCC 33695	Not detected
Candida albicans	ATCC 14053	Not detected
Cytomegalovirus	ATCC VR-807	Not detected
Epstein-Barr virus	ATCC VR-602	Not detected
Herpes simplex virus 1	ATCC VR-260	Not detected
Herpes simplex virus 2	ATCC VR-734	Not detected
Human Adenovirus 40	ATCC VR-931	Not detected
HPV1	ATCC 45021	Not detected
HPV2	ATCC 45022	Not detected
HPV6	ATCC 45150D	Not detected
HPV11	ATCC 45151D	Not detected
HPV26	Korean isolate	Not detected
HPV34	Korean isolate	Not detected
HPV40	Korean isolate	Not detected
HPV42	Korean isolate	Not detected
HPV43	ATCC 40339	Not detected
HPV44	Korean isolate	Not detected
HPV53	Korean isolate	Not detected
HPV54	Korean isolate	Not detected
HPV61	Korean isolate	Not detected
HPV62	Korean isolate	Not detected
HPV69	Korean isolate	Not detected

Organism	Strain No.	Test Result†
HPV70	Korean isolate	Not detected
HPV71	Korean isolate	Not detected
HPV72	Korean isolate	Not detected
HPV73	Korean isolate	Not detected
HPV81	Korean isolate	Not detected
HPV82	Korean isolate	Not detected
HPV83	Korean isolate	Not detected
HPV84	Korean isolate	Not detected
HPV102	Korean isolate	Not detected
HPV16	ATCC 45113D	Detected (HPV16)
HPV18	ATCC 45152D	Detected (HPV18)
HPV31	ATCC 65446	Detected (HPV31)
HPV33	Korean isolate	Detected (HPV33)
HPV35	ATCC 40330	Detected (HPV35)
HPV39	Korean isolate	Detected (HPV39)
HPV45	Korean isolate	Detected (HPV45)
HPV51	Korean isolate	Detected (HPV51)
HPV52	Korean isolate	Detected (HPV52)
HPV56	ATCC 40549	Detected (HPV56)
HPV58	Korean isolate	Detected (HPV58)
HPV59	Korean isolate	Detected (HPV59)
HPV66	Korean isolate	Detected (HPV66)
HPV68	Korean isolate	Detected (HPV68)
SiHa Cell	KCLB 30035	Detected (HPV16)
HeLa Cell	KCLB 10002	Detected (HPV18)

†To prove the availability of the results, the experiment was repeated three times.

※ ATCC: American Type Culture Collection
KCTC: Korean Collection for Type Culture
KCLB: Korean Cell Line Bank

2. Sensitivity

In order to determine the sensitivity of Anyplex™ II HPV HR Detection, a standard serial dilution has been set up from 10^5 to 10^0 copies/reaction plasmid DNA and from 5 x 10^3 to 10^1 cells/mL SiHa cell (HPV16) and HeLa cell (HPV18) and analyzed with Anyplex™ II HPV HR Detection. Detection limit for Anyplex™ II HPV HR Detection was 50 copies/reaction for plasmid DNA and 500 cells/mL for SiHa cell (HPV16) and HeLa cell (HPV18).

3. Reproducibility

A criterion for reproducibility test is to obtain the same results over time. The percent (%) agreement with expected result should be over 95%. Reproducibility test using cloned pDNAs was tested with 3 different product lots, 3 different experimenters, 3 different laboratory sites, and 7 different time points. The overall agreement for the Anyplex™ II HPV HR Detection was 99.4%.

4. Interfering substances

Interference testing was carried out using human whole blood and cervical mucus as external materials not related with target species. Anyplex™ II HPV HR Detection showed clear results that there is no influence on results observed under conditions mentioned above.

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KEY TO SYMBOLS

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

ORDERING INFORMATION

Cat. No.	Product	Size
Anyplex™ II HPV Series		
HP7E00X	**Anyplex™ II HPV HR Detection**	**100 rxns**
HP10380Z	**Anyplex™ II HPV HR Detection**	**25 rxns**
HP7S00X	Anyplex™ II HPV28 Detection	100 rxns
HP10379Z	Anyplex™ II HPV28 Detection	25 rxns
Seeplex® HPV Series		
HP6401Y	Seeplex® HPV4A ACE Screening	50 rxns
Accessory products		
SG1701	Ribo_spin vRD(Viral RNA/DNA Extraction Kit)	50 preps
Automated extraction system		
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
EX00013C	STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384T / 1box
EX00003P	STARMag 96 UniPlate	96 T / 1box
EX00004T	STARMag 96 UniTube	96 T / 1box
EX00036P	STARMag™ S96H N kit	480T / 1box
EX00037P	STARMag™ S96H N kit	960T / 1box
SG71100	SEEPREP32	EA
EX00009P	STARMag 96 ProPrep (Plate Type)	96T / 1box
EX00009T	STARMag 96 ProPrep (Tube Type)	96T / 1box
EX00017P	STARMag 96 ProPrep C (Plate Type)	96T / 1box
EX00017T	STARMag 96 ProPrep C (Tube Type)	96T / 1box
6600532-01	Vial Cap Management System	EA
SG72100	AIOS	EA