For Professional Use Only

Allplex™

STI Essential Assay

(Cat. No.SD9801X, SD10245Z)

A multiplex real-time PCR assay for detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), and *Trichomonas vaginalis* (TV) from urine, genital swab, liquid based cytology specimens, semen, oropharyngeal (throat) swab, and anorectal swab.

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 2797

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

REF **SD9801X** Σ **100** REF **SD10245Z** 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.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- **This product is only for use with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, and Seegene STARlet maximum 5 separate runs.**
- **AIOS** combines Seegene STARlet sold by Seegene with real-time PCR equipment (CFX96 Dx, Manufacturer: Bio-Rad) and plate sealer (Manufacturer: SAMICK THK) to form an automated linkage structure of nucleic acid extraction to PCR.
- **This test has been validated for the following specimen types: urine, genital swab, liquid based cytology specimens, semen, oropharyngeal (throat) swab, and anorectal swab.** 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.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats, and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. Use of 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 prevent any potential splashing or cross-contamination of specimens during preparation.
- Please be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive control. To prevent contamination of the reagents, use of filter tips is recommended.

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

INTENDED USE

Allplex™ STI Essential Assay is a qualitative *in vitro* test for single or multiple detections of *C. trachomatis* (CT), *N. gonorrhoeae* (NG), *M. genitalium* (MG), *M. hominis* (MH), *U. urealyticum* (UU), *U. parvum* (UP), and *T. vaginalis* (TV) from urine, genital swab, liquid based cytology specimens, semen, oropharyngeal (throat) swab, and anorectal swab.

PRINCIPLES AND PROCEDURE OVERVIEW

1. Principles

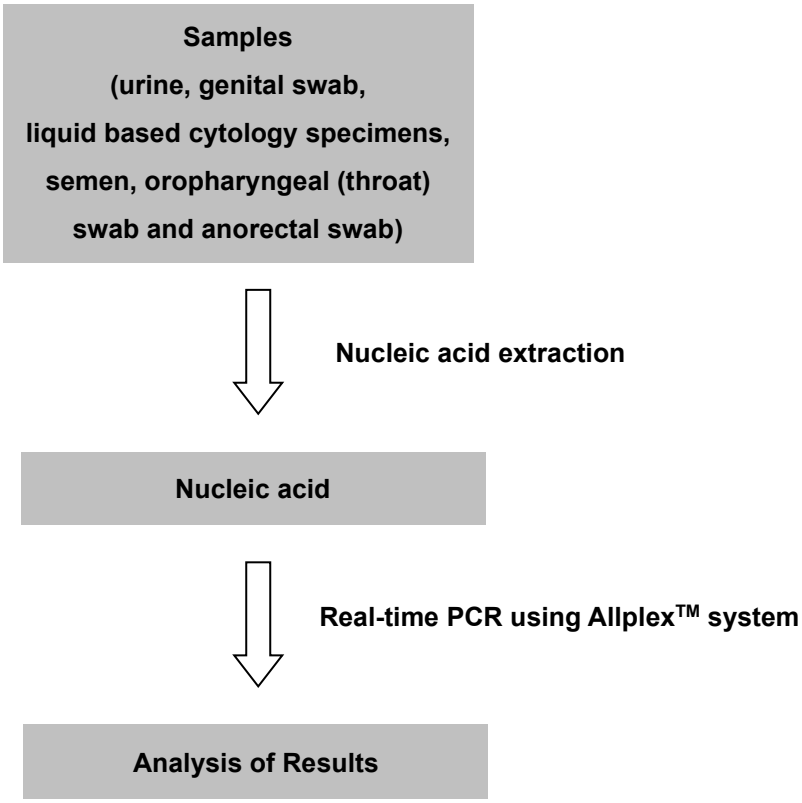
Allplex™ STI Essential Assay exhibits Seegene's proprietary MuDT™ technology, which allows to provide multi-Ct (threshold cycle) values in a single fluorescence channel without melt curve analysis on real-time PCR instrument.

Allplex™ STI Essential Assay is a real-time PCR assay that permits simultaneous amplification and detection of target nucleic acids of *C. trachomatis* (CT), *N. gonorrhoeae* (NG), *M. genitalium* (MG), *M. hominis* (MH), *U. urealyticum* (UU), *U. parvum* (UP), *T. vaginalis* (TV), and Internal Control (IC).

In Allplex™ STI Essential Assay, an endogenous human gene is used as Internal Control (IC) for monitoring the whole process from sample collection to nucleic acid extraction as well as to check for any possible PCR inhibition. PCR efficiency may be reduced by inhibitors that may be present in clinical specimens. However, due to the inconsistencies in the number of human cells contained in urine and anorectal swab, IC is exogenously added only to urine and anorectal samples to serve as an exogenous overall process control. IC is co-amplified with target nucleic acids within the clinical specimen. To prevent amplification product acting as potential contaminants, Uracil-DNA glycosylase (UDG) system is employed in Allplex™ STI Essential Assay.

The natural function of UDG is to prevent mutagenesis by eliminating uracil from DNA molecules by cleaving N-glycosylic bond and initiating base-excision repair (BER) pathway. Therefore, UDG systems are used to control cross-contamination of samples with amplicons.

2. Procedure Overview



BACKGROUND INFORMATION

The term sexually transmitted diseases (STDs) is used to refer to a variety of clinical syndromes caused by pathogens that can be acquired and transmitted through sexual activity.

More than 30 bacterial, viral, and parasitic pathogens are transmissible sexually and constitute a group of infections called to as sexually transmitted infections (STIs).

Some STIs can increase the risk of HIV acquisition three-fold or more. STIs can have serious consequences beyond the immediate impact of the infection itself, through mother-to-child transmission of infections and chronic diseases.

More than 1 million people acquire an STI every day. Each year, an estimated 500 million people become ill with one of 4 STIs: chlamydia, gonorrhoea, syphilis, and trichomoniasis.

1. *Chlamydia trachomatis*

Chlamydia trachomatis, the etiological agent of chlamydia, causes substantial morbidity and economic cost worldwide.

Chlamydial infections in women are usually asymptomatic. However, these can result in pelvic inflammatory disease (PID), which is a major cause of infertility, ectopic pregnancy, and chronic pelvic pain. As with other inflammatory STDs, chlamydial infection might facilitate the transmission of human immunodeficiency virus (HIV) infection. In addition, pregnant women infected with chlamydia can pass the infection to their infants during delivery, potentially resulting in neonatal ophthalmia and pneumonia.

2. *Neisseria gonorrhoeae*

Gonorrhea is a very common infectious disease. Most women with gonorrhea are asymptomatic. If undetected, not treated, or inappropriately treated, infection can ascend to the upper genital tract and cause complicated gonococcal infection (e.g. PID and related sequelae such as ectopic pregnancy and infertility) in women, and penile oedema and epididymitis in men.

3. *Trichomonas vaginalis*

Trichomonas vaginalis is the etiological agent of the most prevalent non-viral STI worldwide. *T. vaginalis* may cause an abnormal vaginal discharge (trichomoniasis) in women and may be responsible for as much as 10~12% of non-gonococcal urethritis cases in men, the infection may be asymptomatic in at least 50% of women and 70~80% of men.

4. Genital mycoplasmas

M. genitalium and *M. hominis* and the two ureaplasma species *U. urealyticum* (previously known as *U. urealyticum*, biovar 2) and *U. parvum* (previously known as *U. urealyticum*, biovar 1) are commonly found in the human urogenital tract.

M. genitalium was first identified in the early 1980s and has been recognized as a cause of male urethritis, responsible for approximately 15~20% of nongonococcal urethritis (NGU) cases, 20%~25% of nonchlamydial NGU, and approximately 30% of persistent or recurrent urethritis. *M. genitalium* is found in the cervix and/or endometrium of women with PID more often than in women without PID.

Ureaplasmas can be found in the cervix or vagina of 40~80% of sexually active, asymptomatic women, and *M. hominis* in 20~50%. Accordingly, ureaplasmas and *M. hominis* should be considered primarily as commensals when detected in the lower genital tract. Although there is an ongoing debate, evidence that these microbes cause lower genital tract diseases, including cervicitis, in women is accumulating. The accurate diagnosis of *Ureaplasma* spp. and *Mycoplasma hominis* in cervical samples is important because these microorganisms could be pathogenic and could be associated with adverse pregnancy outcomes, postpartum sepsis, neonatal systemic inflammatory response syndrome, and bronchopulmonary dysplasia.

The current standard of care for clinical sexually transmitted infection (STI) screening involves the use of separate tests to detect the presence of each possible pathogen. Most commercially available tests only focus on detecting the two most prevalent bacterial causes of STIs: CT and NG. However, since most STIs do not show noticeable symptoms, it is a key to screen for a wider range of pathogens. Further complicating STI diagnosis is that different pathogens can cause similar symptoms, but the antibiotic treatment regimen may differ depending upon the pathogen. This complexity of issues makes simultaneous and accurate STI detection a major key to cost-effective patient care.

REAGENTS

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

Order information (REF SD9801X)

Allplex™ STI Essential Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	4X STI-EA MOM	500 µL	MuDT Oligo Mix (MOM): - Amplification and detection reagent
PREMIX	EM1	500 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	STI-EA PC	50 µL	Positive Control (PC) - Mixture of pathogen clones
CONTROL IC	ASTI IC	1,000 µL	Internal Control (IC) for urine and anorectal swab specimen
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 SD10245Z)

Allplex™ STI Essential Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	4X STI-EA MOM	125 µL	MuDT Oligo Mix (MOM): - Amplification and detection reagent
PREMIX	EM1	125 µL	- DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	STI-EA PC	50 µL	Positive Control (PC) - Mixture of pathogen clones
CONTROL IC	ASTI IC	250 µL	Internal Control (IC) for urine and anorectal swab specimen
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 Allplex™ STI Essential Assay should be stored at ≤ -20°C. All components are stable under recommended storage conditions until the expiry date stated on the label. This product can be used for 30 days after initial opening of the kit and performance is not affected for up to 5 freezing and thawing cycle. If the reagents are to be used only intermittently, they should be stored in aliquots.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and Sterile pipette tips
- 1.5 mL microcetrifuge tubes
- Ice Maker
- Desktop centrifuge
- Mini plate spiner Centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection system (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Cat. No. HSP9655, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Cat. No. HSP9955, Bio-Rad)
- 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) *
- Saline solution
- Clean bench

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

PROTOCOL

1. Specimen Collection, Storage, and Transport

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

Urine specimen
Genital swab specimen
Liquid based cytology specimen
Semen
Oropharyngeal (throat) swab specimen
Anorectal swab specimen

Note: To ensure high sample quality, specimens should be transported as fast as possible. The specimens should be transported at indicated temperatures.

A. Specimen Collection

Urine specimen

- The patient should be advised not to urinate for at least two hours prior to specimen collection.
- Collect 10~30 mL of first-catch urine in a clean container of polypropylene. Close and label the sample containers. Strictly adhere to the instructions given for storage and transport.

Genital swab, Oropharyngeal (throat) swab and Anorectal swab specimen

For the collection of genital swab, oropharyngeal (throat) swab, and anorectal swab specimens, please use following materials :

- Genital swab, oropharyngeal (throat) swab and anorectal swab specimens can be collected and transported in 1~3 mL of the following mediums :
  - ENAT PM 2ML REGULAR APPLICATOR (Copan)
  - UTM with Flocked Swabs (Copan)

  Note: Oropharyngeal (throat) swab and anorectal swab specimen have not been validated with the UTM with Flocked Swabs (Copan).

- Leave the swab in the transport medium. Close and label the sample container. Strictly adhere to the instructions given for storage and transport.
- When using genital swabs, follow a recommended protocol to collect columnar and squamous epithelium cells after removal of the cervical mucus.

Liquid based cytology specimen

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

Semen

- Collect semen in a clean container of polypropylene. Close and label the sample container. Strictly adhere to the instructions given for storage and transport.

B. Specimen Storage & Transport

<table>
<tr><th colspan="2" rowspan="2">Specimen</th><th colspan="2">Storage & Transport</th><th rowspan="2">Note</th></tr>
<tr><th>Temp.</th><th>Duration*</th></tr>
<tr><td colspan="2">Urine specimen</td><td>2~8℃</td><td>1 week</td><td rowspan="10">- Performance may be affected by prolonged storage of specimens.
- Specimens should also adhere to local and national instructions for transport of pathogenic material.</td></tr>
<tr><td colspan="2">Genital swab specimen</td><td>2~8℃</td><td>1 week</td></tr>
<tr><td rowspan="3">Liquid based cytology</td><td>ThinPrep® medium</td><td rowspan="2">2~8℃** & Room Temperature**</td><td rowspan="2">90 days</td></tr>
<tr><td>CellPreserv</td></tr>
<tr><td>SurePath™ medium</td><td>2~8℃</td><td>2 weeks</td></tr>
<tr><td colspan="2">Semen</td><td>2~8℃</td><td>1 week</td></tr>
<tr><td colspan="2">Oropharyngeal (throat) swab</td><td>2~8℃</td><td>3 days</td></tr>
<tr><td colspan="2" rowspan="2">Anorectal swab</td><td>2~8℃</td><td>2 days</td></tr>
<tr><td>-20℃</td><td>1 month</td></tr>
</table>

* Duration: The time period from specimen collection to test including specimen storage and transport prior to the test.
** Optimum temperature for transport is 2~25℃.

2. Nucleic Acid Extraction

A. Pre-treatment of specimen

Note: The pre-treatment process for nucleic acid extraction is the same for both manual and automated extraction system.

Genital swab, Oropharyngeal (throat) swab, and Anorectal swab specimens

- Genital swab, oropharyngeal (throat) swab, and anorectal swab specimen is used without pre-treatment.

Note: Oropharyngeal (throat) swab and Anorectal swab specimens have not been validated with SEEPREP32.

Urine & Liquid based cytology specimens

- Equilibrate samples in the room temperature (19~25℃).
- Centrifuge 1 mL of urine and Liquid based cytology specimen for 15 minutes at 15,000 x *g* (13,000 rpm).
- After discarding supernatant, pellet must be resuspended in Saline solution at recommended volume (See Recommended Vol. of 2.C-1, 2.C-2) by thoroughly vortexing.

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

Note: Urine, 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 does not require a pre-treatment step.

Note: SurePath™ used after pre-treatment has not been validated with the STARMag 96 X 4 Viral DNA/RNA 200 C kit.

Note: SurePath™ have not been validated with Ribo_spin vRD kit, NucliSENS® easyMAG®, and SEEPREP32.

- Follow the manufacturer’s protocol.

Semen

- Equilibrate semen for 30 min in darkness until liquefaction.in the room temperature (19~25℃).
- Dilute three times with Saline solution at recommended volume (See Recommended Vol. Of 2.C) by thoroughly vortexing.

Note: Semen have not been validated with SEEPREP32.

- Follow the manufacturer's protocol.

B. Internal Control

Note: For other specimens, except urine and anorectal swab specimen, endogenous gene is used for internal control. Therefore it does not require additional IC included in the kit.

Note: The ASTI IC is included in the kit. This allows the user to confirm not only the nucleic acid extraction procedure, but also identify any PCR inhibition.

- For urine and anorectal swab specimen, 10 µL of the ASTI IC must be added to each specimen before the nucleic acid extraction.

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

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

** SurePath™ used after pre-treatment has not been validated with the STARMag 96 X 4 Viral DNA/RNA 200 C kit.

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

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

** SurePath™ used after pre-treatment has not been validated with the STARMag 96 X 4 Viral DNA/RNA 200 C kit.

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

* SurePath™ used after pre-treatment has not been validated with the STARMag 96 X 4 Viral DNA/RNA 200 C kit.

C-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

* SurePath™ used after pre-treatment has not been validated with the STARMag 96 X 4 Viral DNA/RNA 200 C kit.

C-5. NucliSENS® easyMAG®

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

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
NucliSENS® easyMAG®	BioMerieux	200111	Specimen: 200 μL* Magnetic Silica: 50μL Elution: 100 μL

* In case of Urine specimen, resuspend the pellet with 200 μL of saline solution and add 10 μL of ASTI IC.

* In case of anorectal swab specimen, add 10 μL of ASTI IC.

Note: SurePath™ has not been validated with NucliSENS® easyMAG®.

C-6. SEEPREP32

- 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
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

* In case of Urine specimen, resuspend the pellet with 200 μL of saline solution and add 10 μL of ASTI IC.

Note: Semen, Oropharyngeal (throat) swab, and Anorectal swab specimens have not been validated with SEEPREP32.

D. Manual Nucleic Acid Extraction Kits

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

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

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

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

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

**** In case of urine specimen, resuspend the pellet with 190 μL of saline solution and add 10 μL of ASTI IC.

**** In case of anorectal swab specimen, add 10 μL of ASTI IC.

E. Summary

specimen	Automation Extraction System							Manual Extraction kits	
	Microlab NIMBUS IVD / STARlet IVD		Seegene NIMBUS / STARlet*		SEEPREP32		NucliSENS® easyMAG®	QIAamp® DSP DNA Mini Kit / DNA Mini Kit	Ribo_ spin vRD
	Universal Cartridge Kit	Viral DNA/RNA 200 C Kit	Universal Cartridge Kit	Viral DNA/RNA 200 C Kit	ProPrep	ProPrep C			
Urine (w/ pre-treatment)	O	O	O	O	O	O	O	O	O
Urine (w/o pre-treatment)	O	O	O	O	X	X	X	X	X
Genital swab(ENAT)	O	O	O	O	O	O	O	O	O
Genital swab(UTM)	O	O	O	O	O	O	O	O	O
ThinPrep® (w/ pre-treatment)	O	O	O	O	O	O	O	O	O
ThinPrep® (w/o pre-treatment)	O	O	O	O	X	X	X	X	X
SurepathTM (w/ pre-treatment)	O	X	O	X	X	X	X	O	X
SurepathTM (w/o pre-treatment)	O	O	O	O	X	X	X	X	X
CellPreserv	O	O	O	O	O	O	O	O	O
Semen	O	O	O	O	X	X	O	O	O
Oropharyngeal (throat) swab	O	O	O	O	X	X	O	O	O
Anorectal swab	O	O	O	O	X	X	O	O	O

* Optional: AIOS can be used with Seegene STARlet.

3. Preparation for Real-time PCR

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 PCR reactions. Use extreme care to ensure no cross-contamination.

Note: Completely thaw all reagents on ice.

Note: Spin down the reagent tubes to remove drops from inside of the cap.

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

A. Prepare the PCR Mastermix.

5 μL	4X STI-EA MOM
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 over 5 times or quick vortex, and spin down.

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

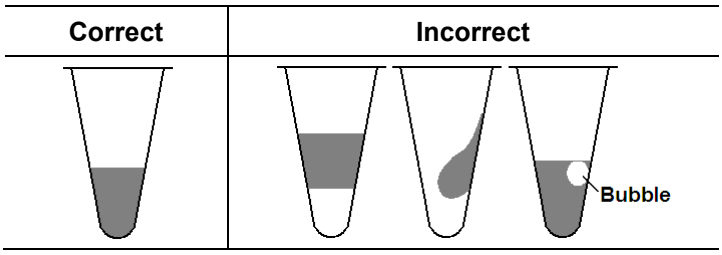
D. Add 5 μL of each sample's nucleic acids into the tube containing the PCR Mastermix.

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

E. Close the cap, and spin down the PCR tubes.

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

Note: The PCR tubes must be centrifuged before running PCR reaction. It needs to force the liquid to the bottom and to eliminate air bubbles.



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 STI-EA PC instead of sample's nucleic acid.

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

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

Note: Use the PX1 PCR plate sealer when using Permanent clear heat seal instead of a cap.

REAL-TIME PCR INSTRUMENT SET UP AND RESULTS ANALYSIS

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

1.1. Real-time PCR Instrument set up

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

A. Protocol Setup

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

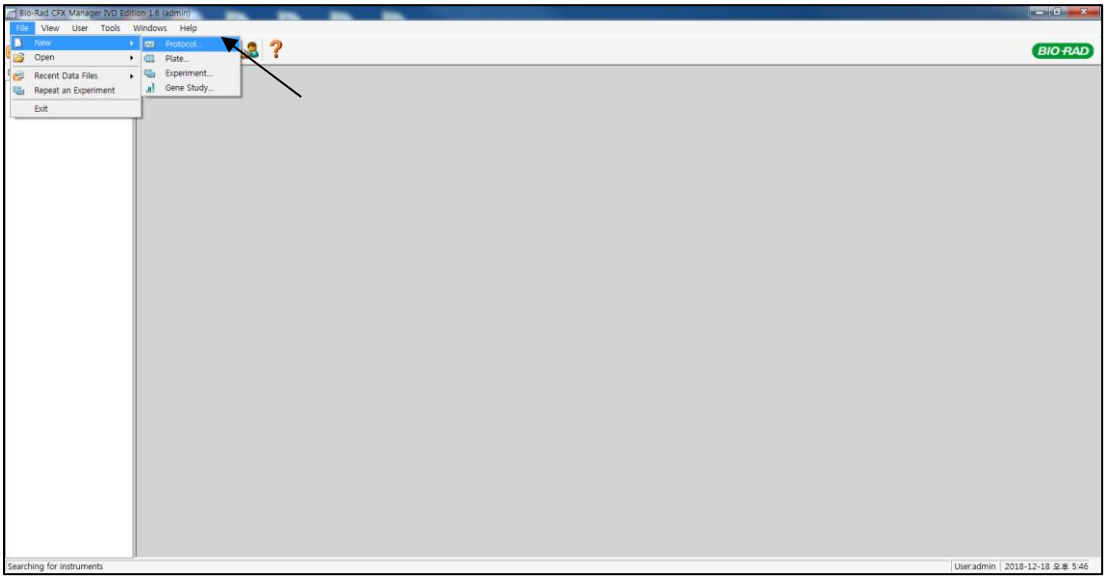


Fig. 1. **Protocol Setup**

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

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

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

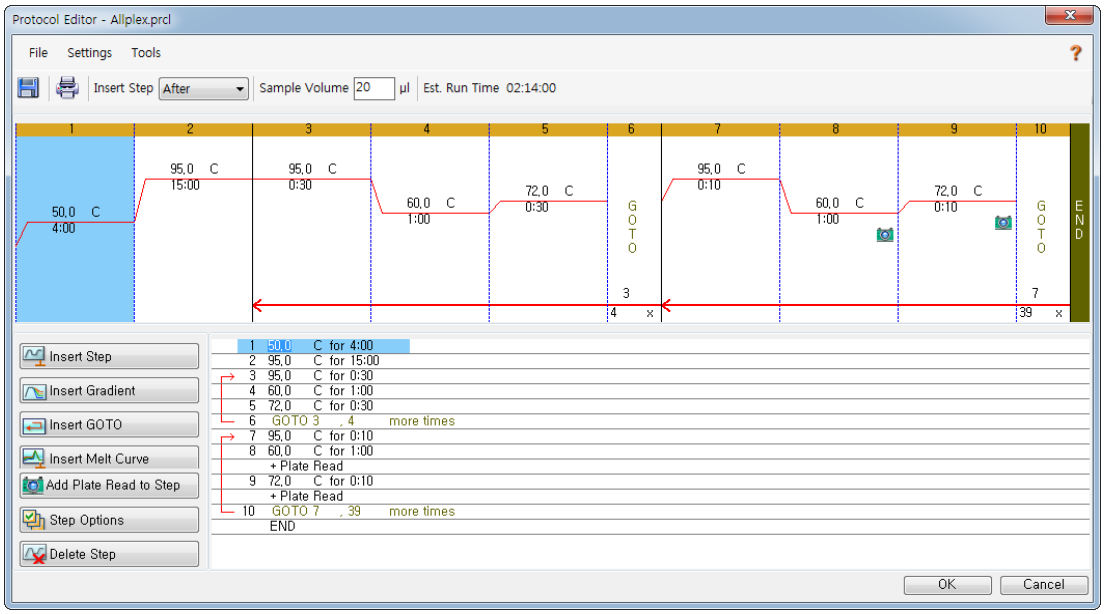


Fig. 2. **Protocol Editor**

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

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

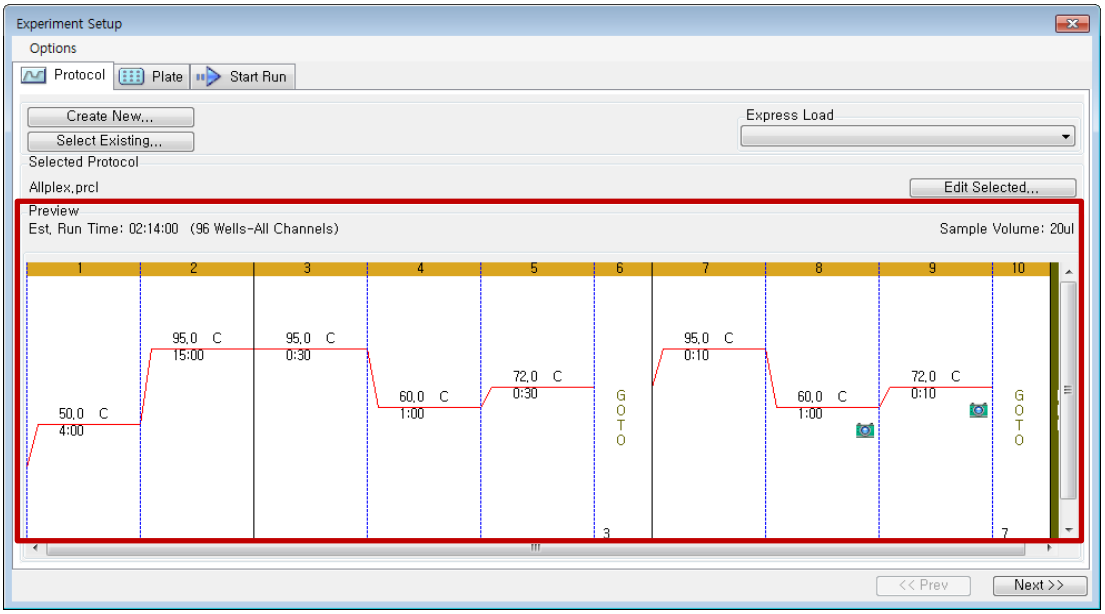


Fig. 3. **Experiment Setup: Protocol**

B. Plate Setup

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

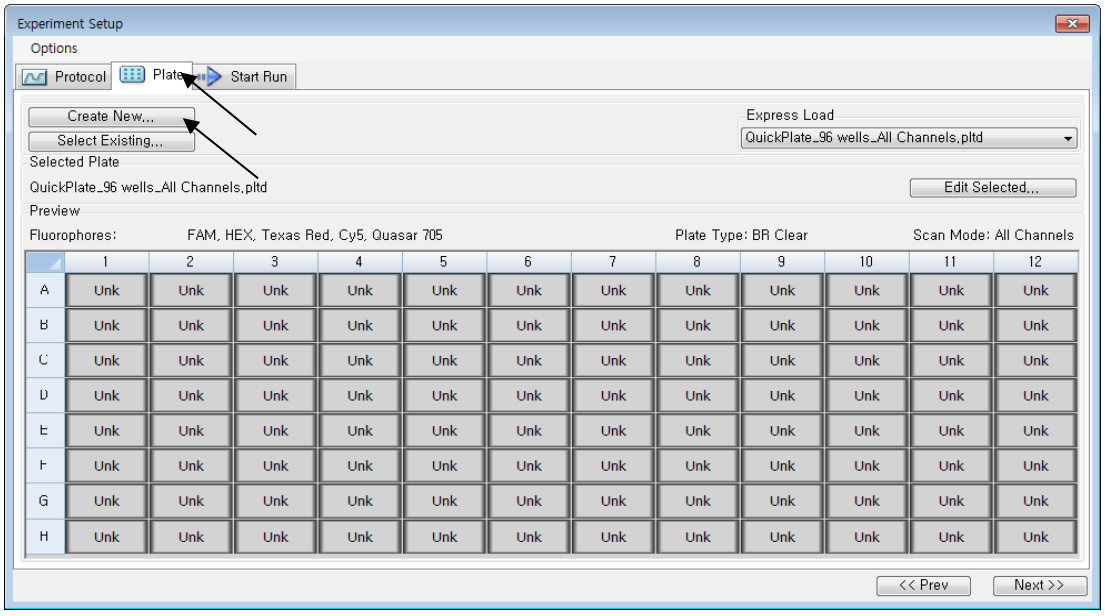


Fig. 4. **Plate Editor**

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

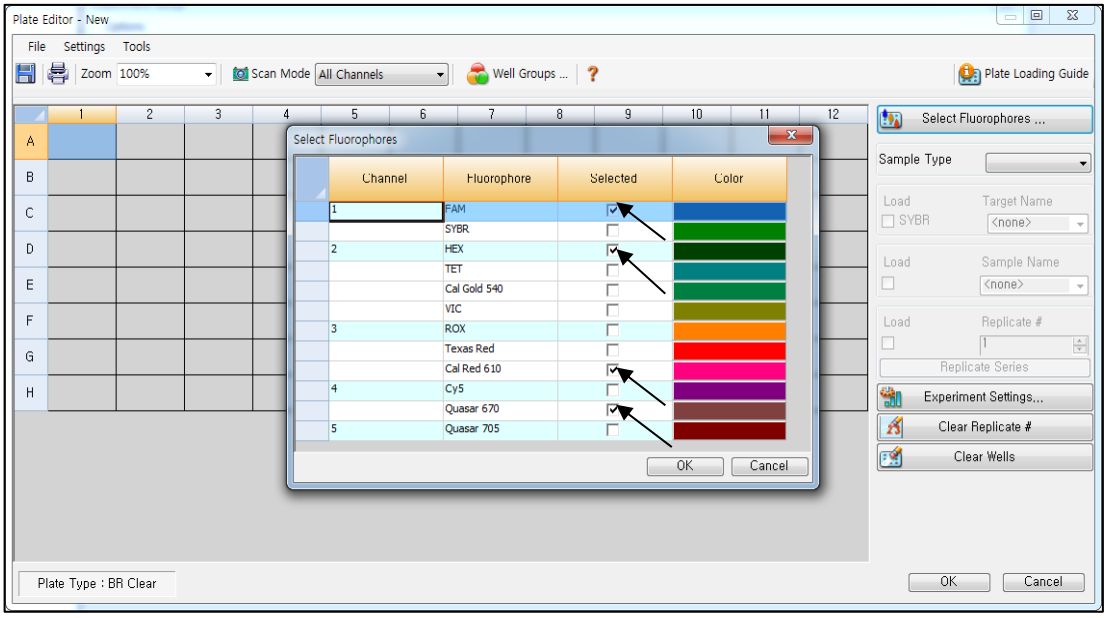


Fig. 5. **Select Fluorophores** (**FAM**, **HEX**, **Cal Red 610**, and **Quasar 670**)

3) 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**, and **Quasar 670**) to specify the fluorophores to be detected in the selected wells.

5) Type the **Sample Name** and press enter key.

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

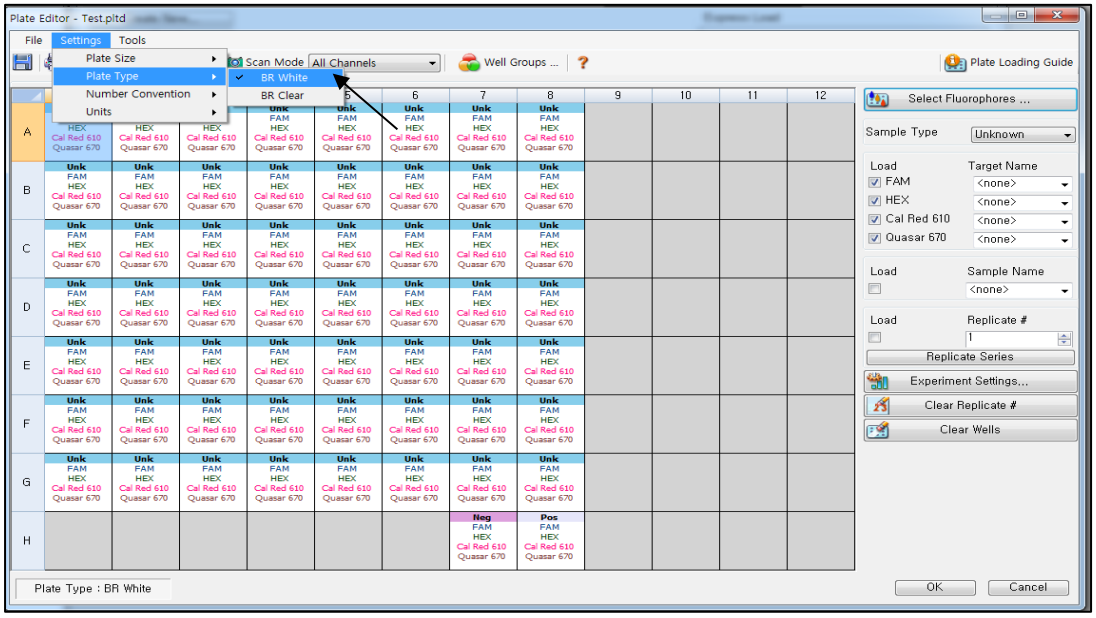


Fig. 6. **Plate Setup**

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

8) Return to the **Experiment Setup** window.

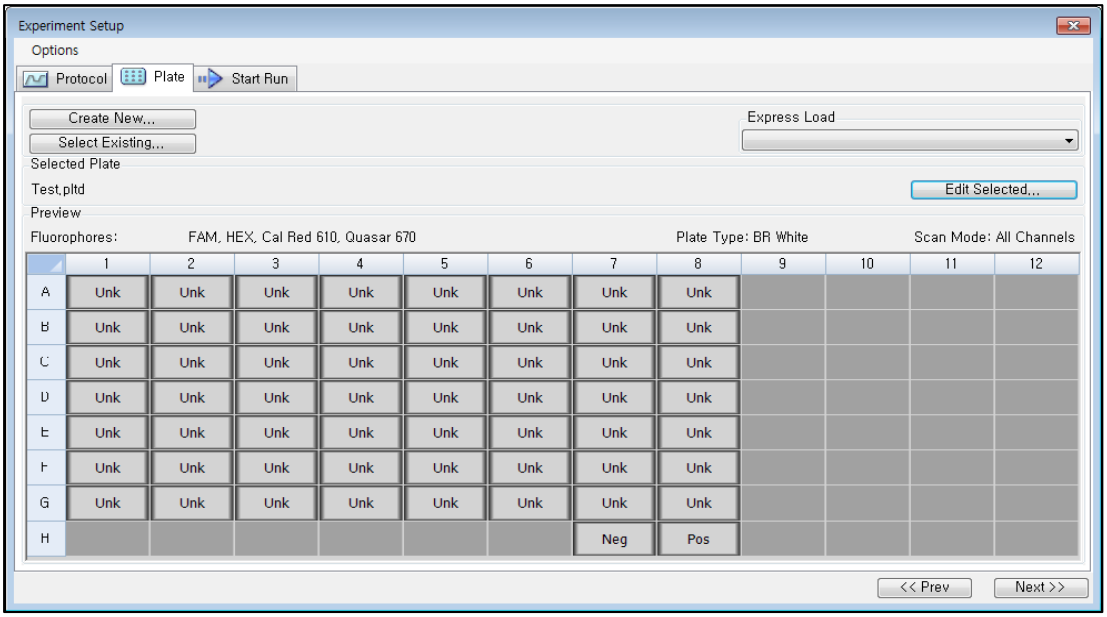


Fig. 7. **Experiment Setup: Plate**

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.

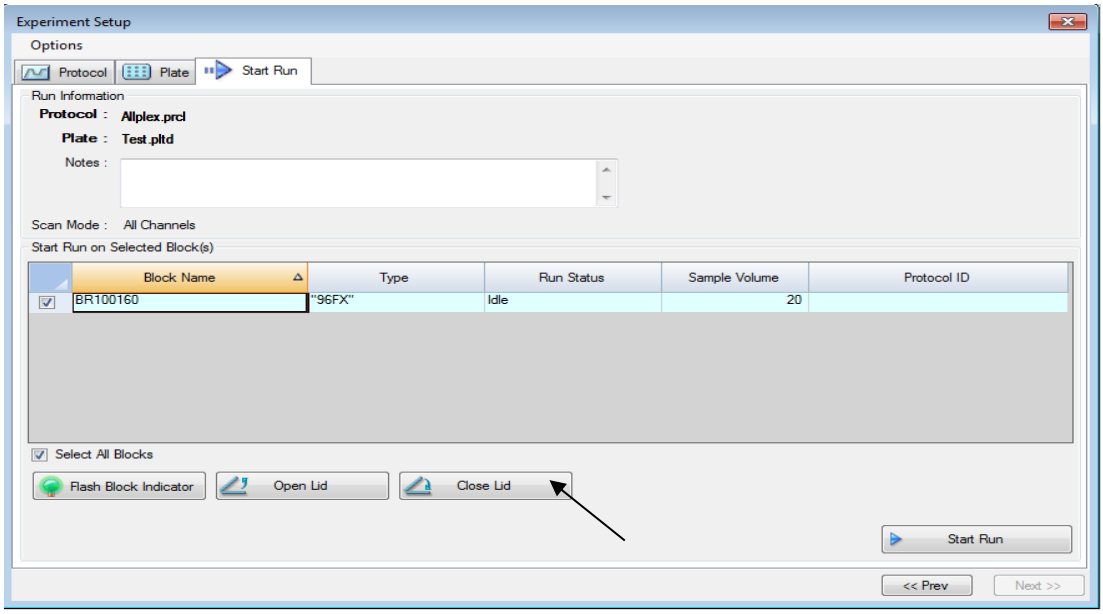


Fig. 8. **Close Lid.**

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 for all of amplification curve detection step from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).

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

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

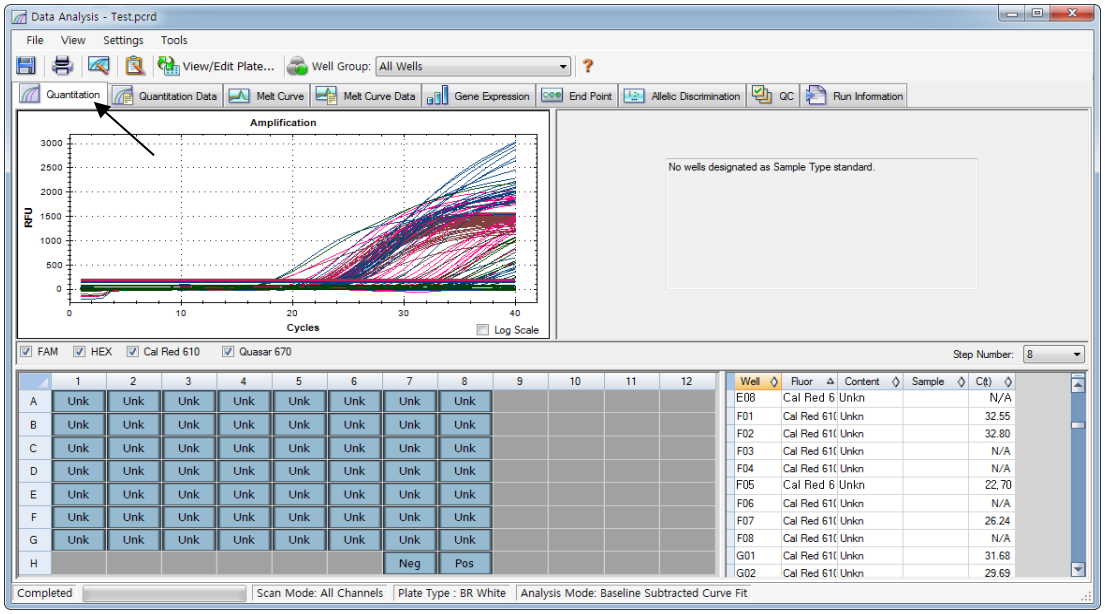


Fig. 9. **Amplification curve results**

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

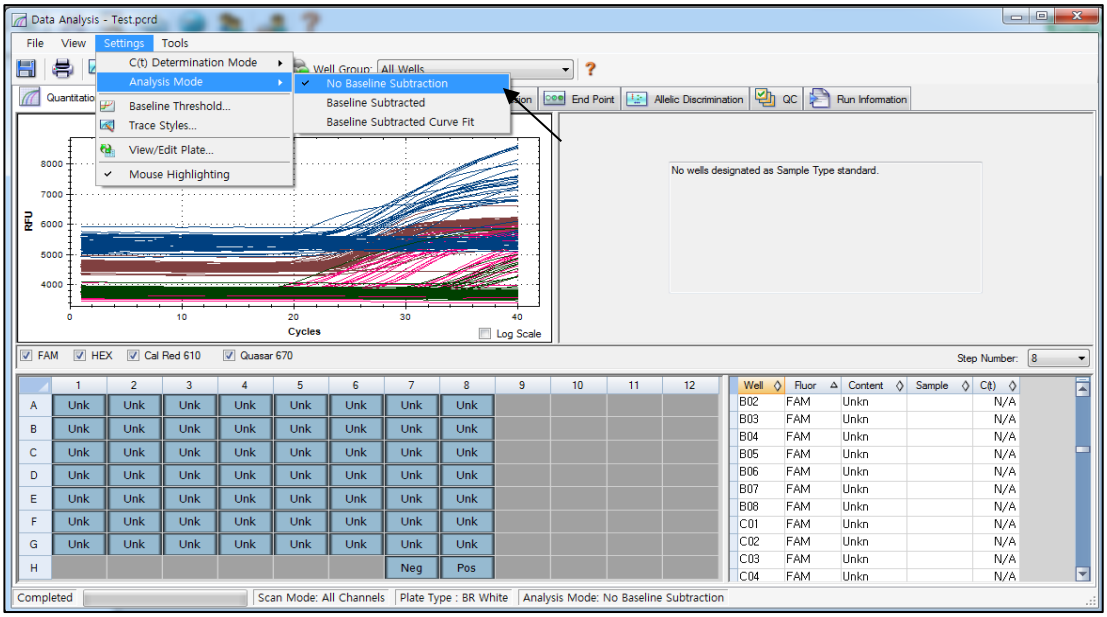


Fig. 10. **No Baseline Subtraction**

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

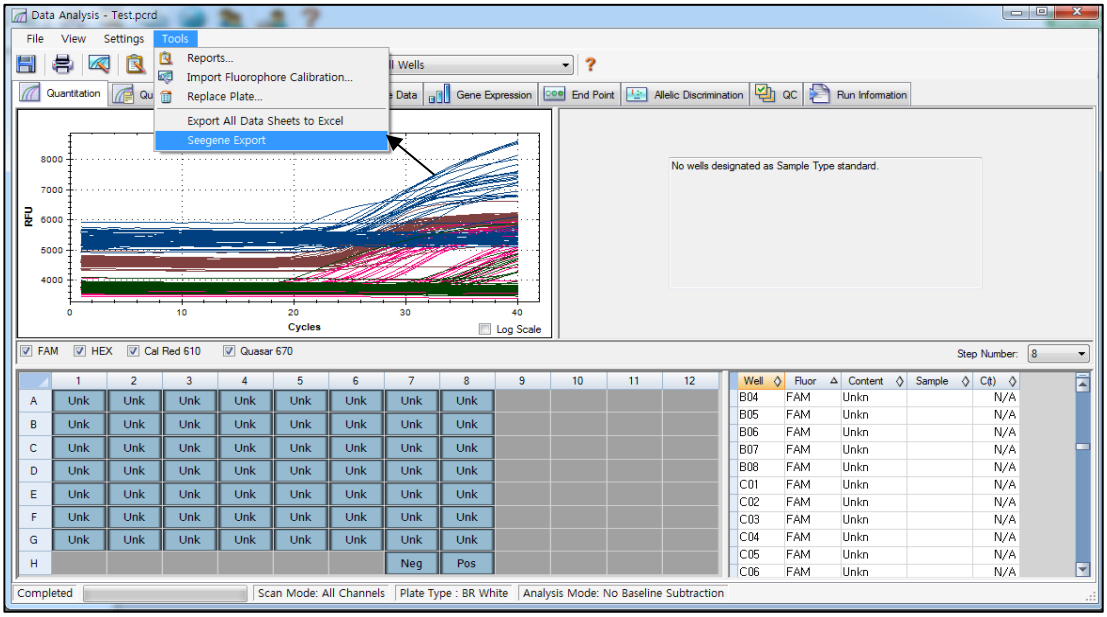


Fig. 11. **Seegene Export**

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

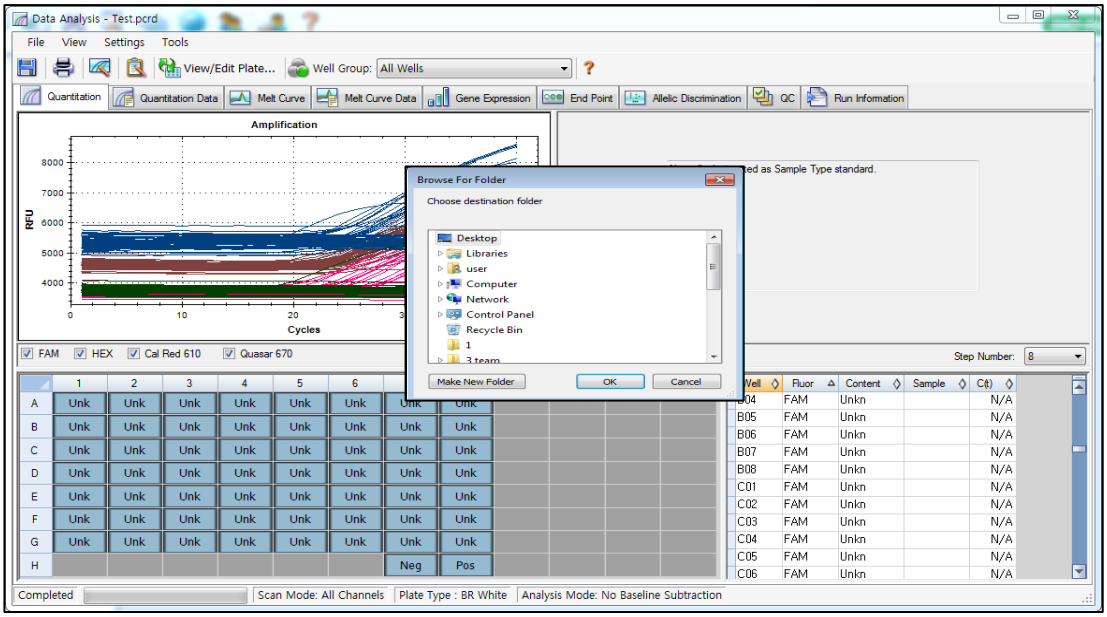


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

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

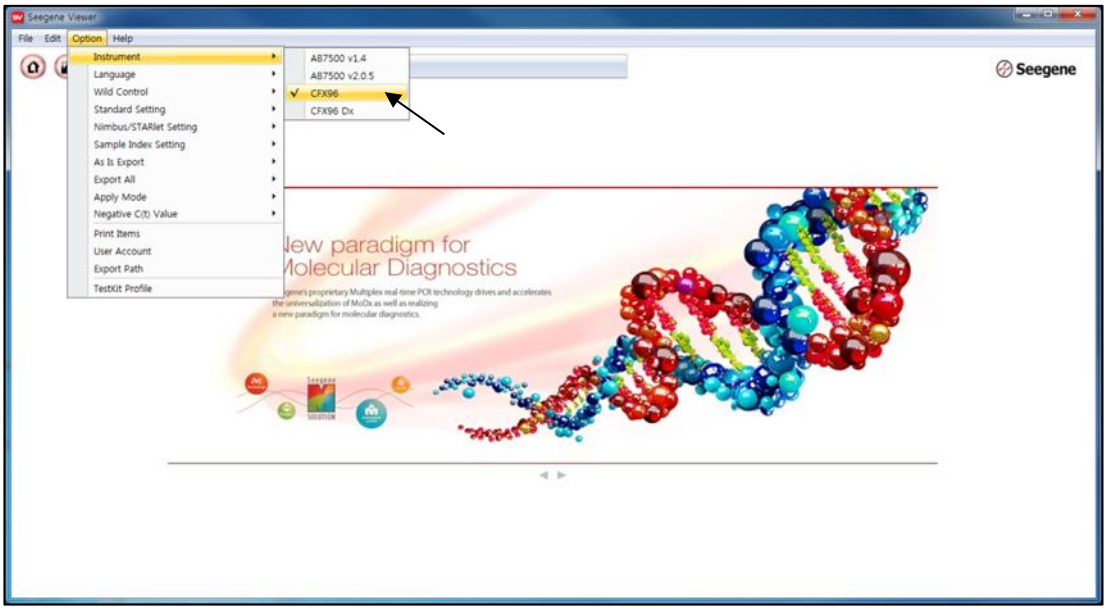


Fig. 13. **Seegene Viewer**

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

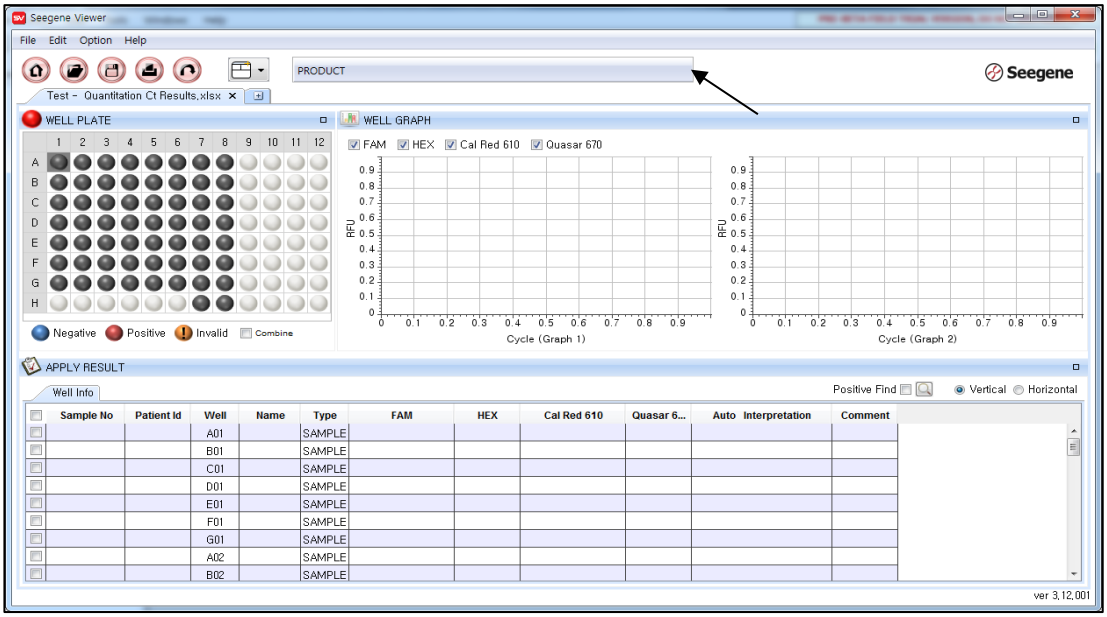


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

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

3) Check the result for each well.

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

4) Validity Criteria of Control Results

a. Valid Assay Run

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

Control	Seegene Viewer Result								
	FAM (Ct)		HEX (Ct)		Cal Red 610 (Ct)		Quasar670 (Ct)		Auto Interpretation
	UU	NG	MH	MG	UP	CT	TV	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

b. Invalid Assay Run

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

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**.

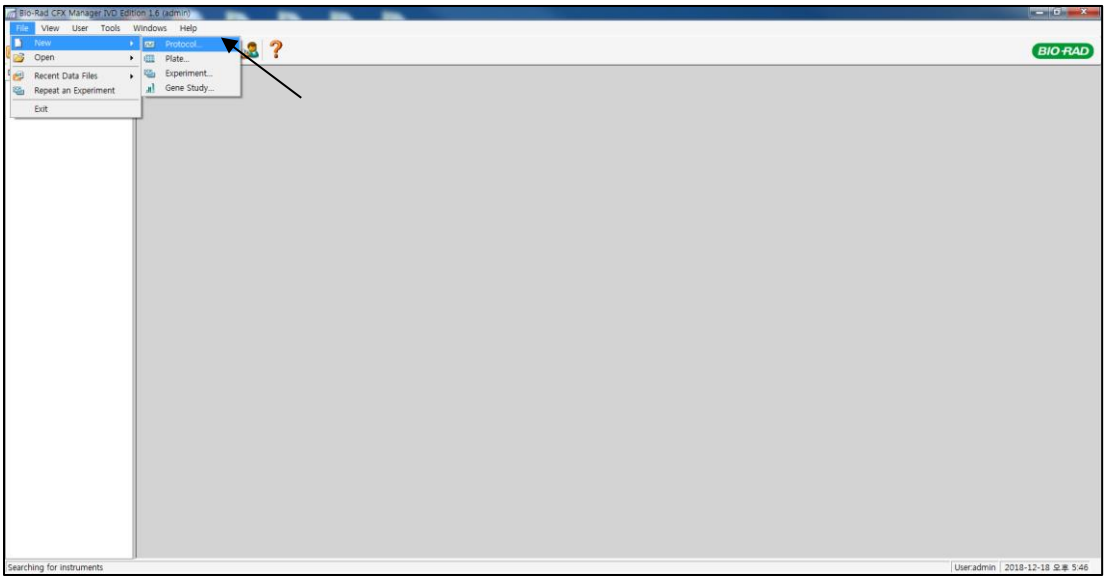


Fig. 1. **Protocol Setup.** Create a new protocol or load an existing protocol for the run

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

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

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

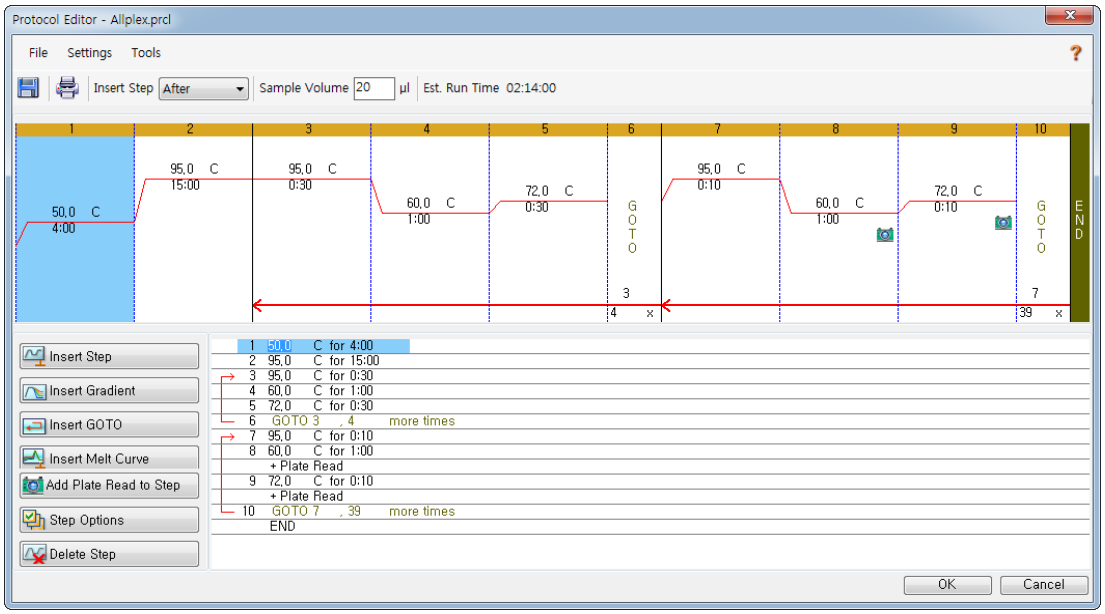


Fig. 2. **Protocol Editor**

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

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

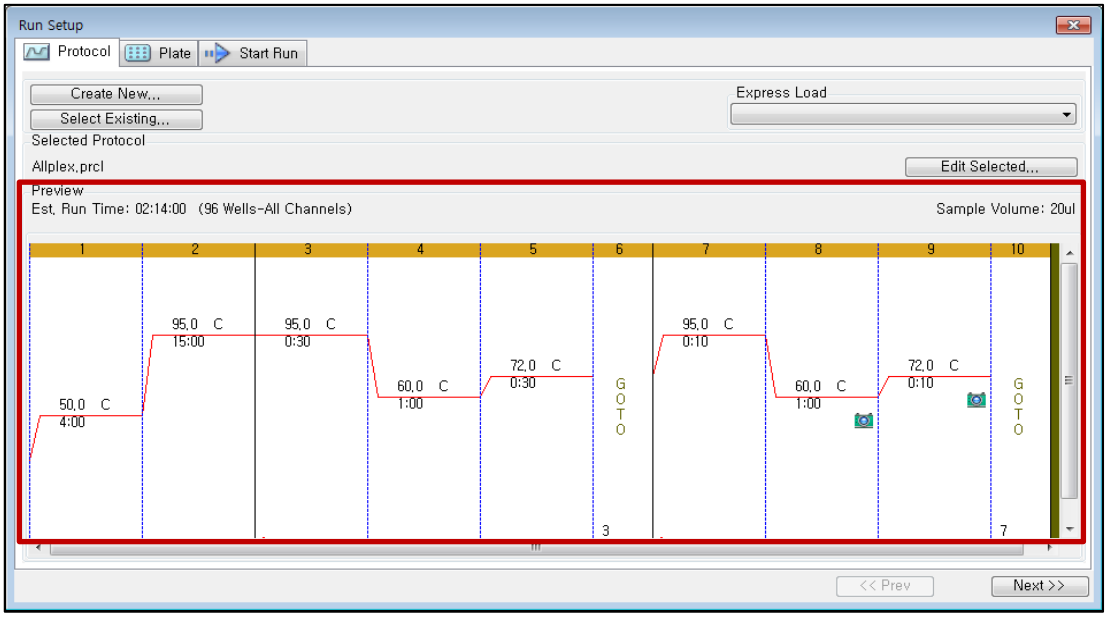


Fig. 3. **Run Setup: Protocol**

B. Plate Setup

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

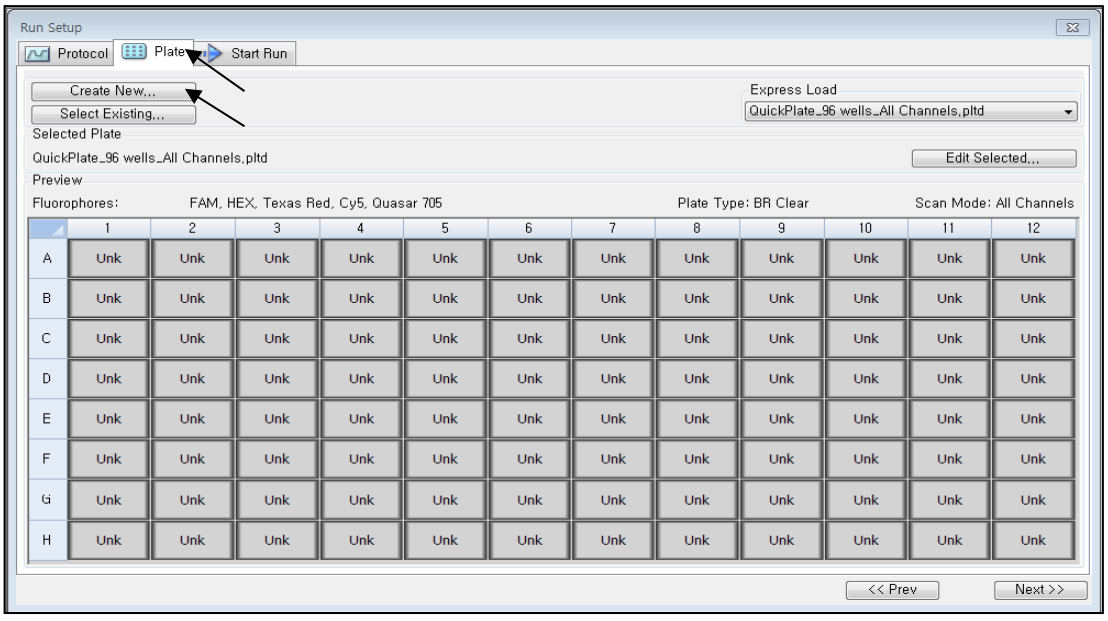


Fig. 4. **Plate Editor**. Create a new plate

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

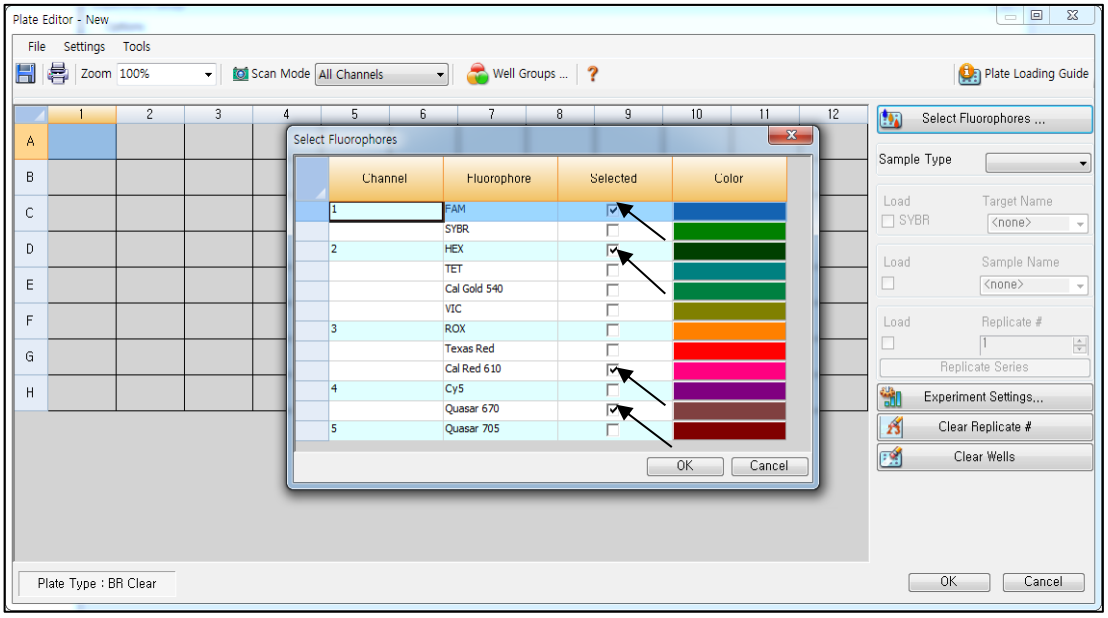


Fig. 5. **Select Fluorophores** (**FAM**, **HEX**, **Cal Red 610**, and **Quasar 670**)

3) 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**, and **Quasar 670**) to specify the fluorophores to be detected in the selected wells.

5) Type the **Sample Name** and press enter key.

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

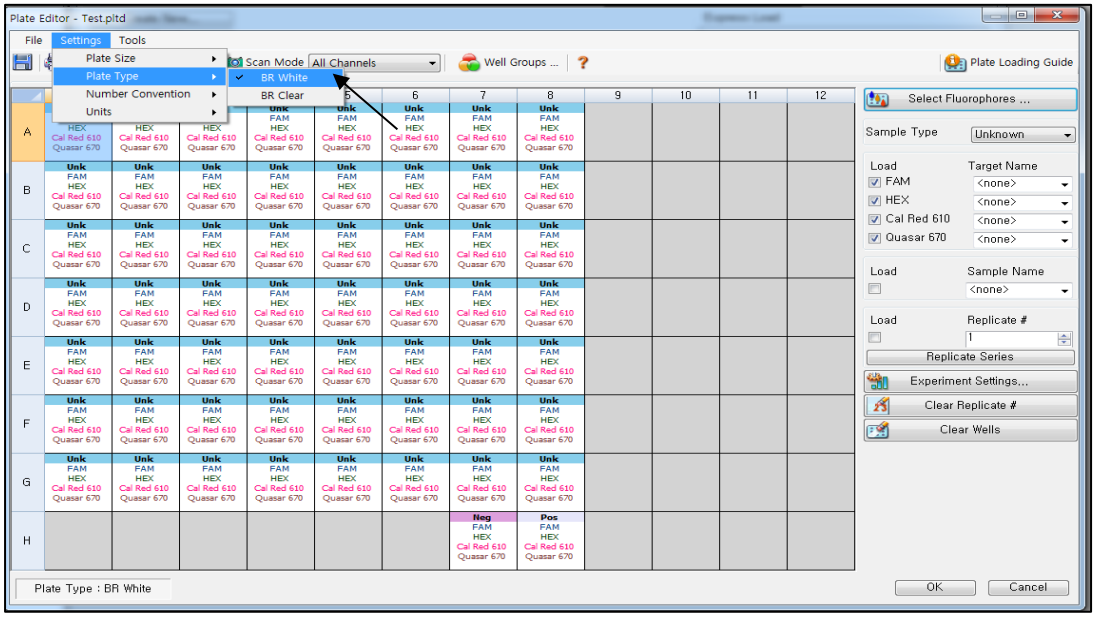


Fig. 6. **Plate Setup**

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

8) Return to the **Run Setup** window.

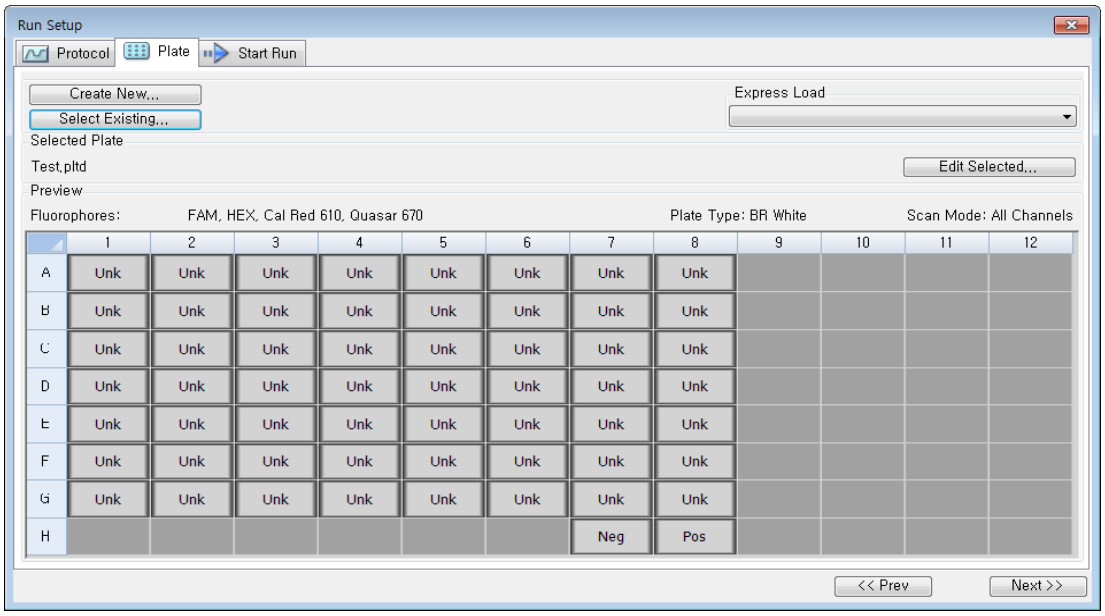


Fig. 7. **Run Setup: Plate**

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.

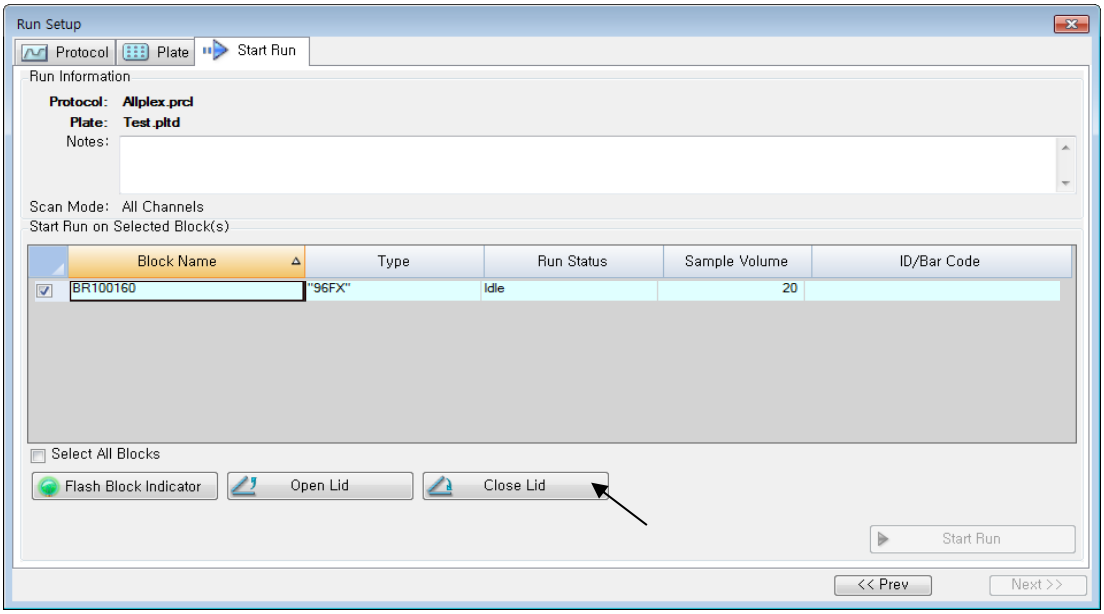


Fig. 8. **Close Lid.**

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

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).

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

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

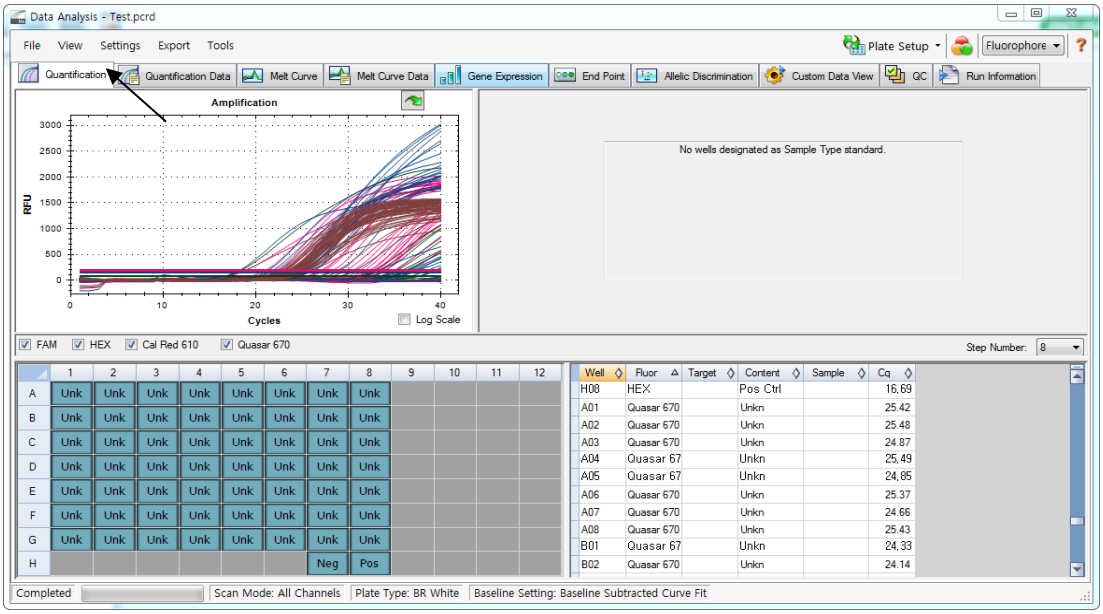


Fig. 9. **Amplification curve results**

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

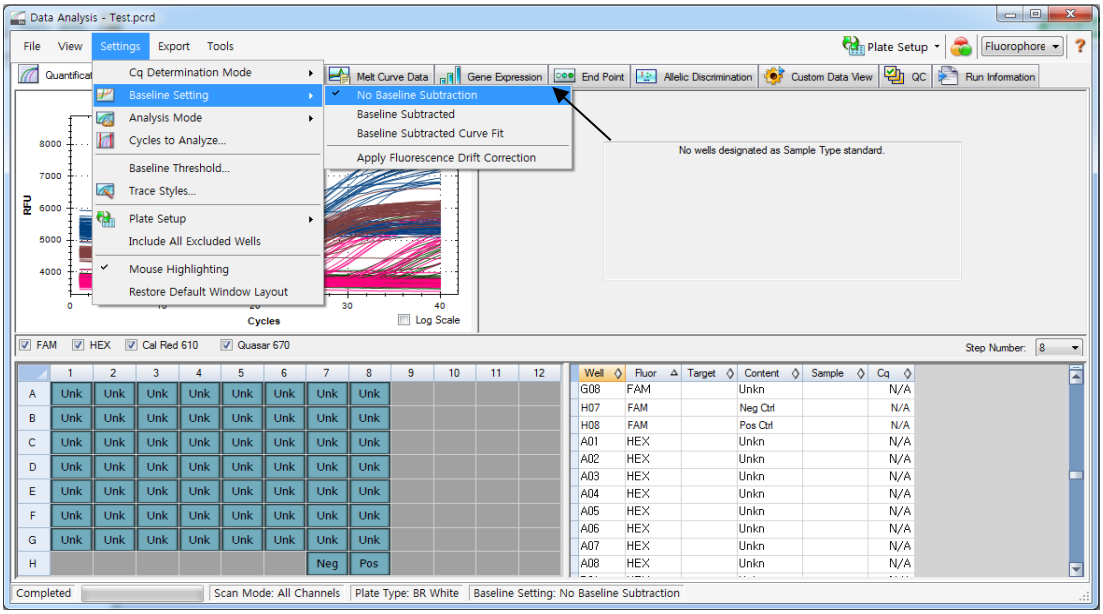


Fig. 10. **No Baseline Subtraction**

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

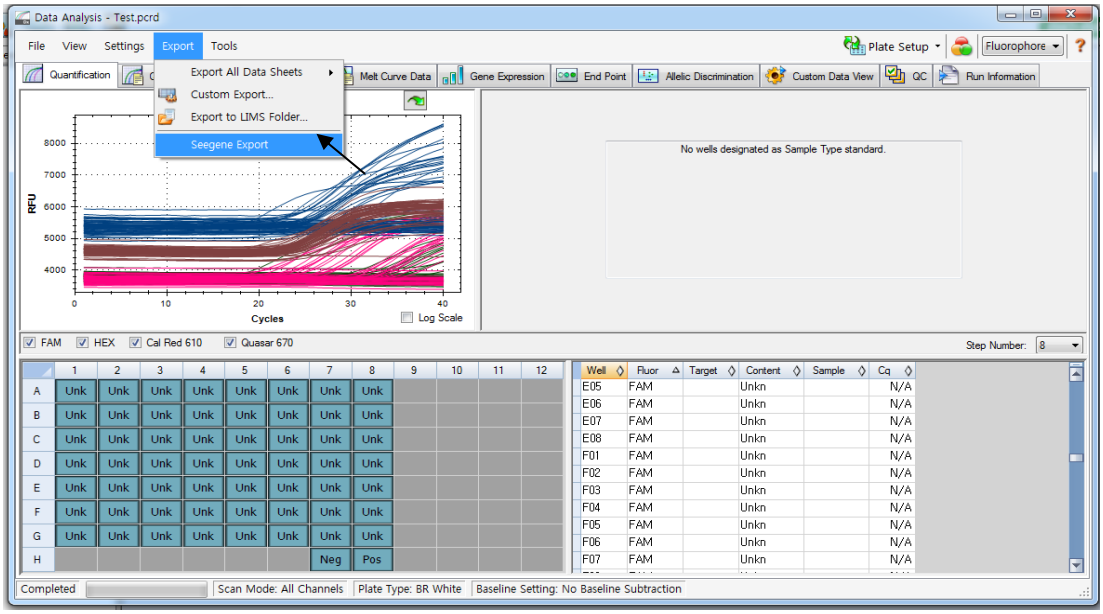


Fig. 11. **Seegene Export**

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

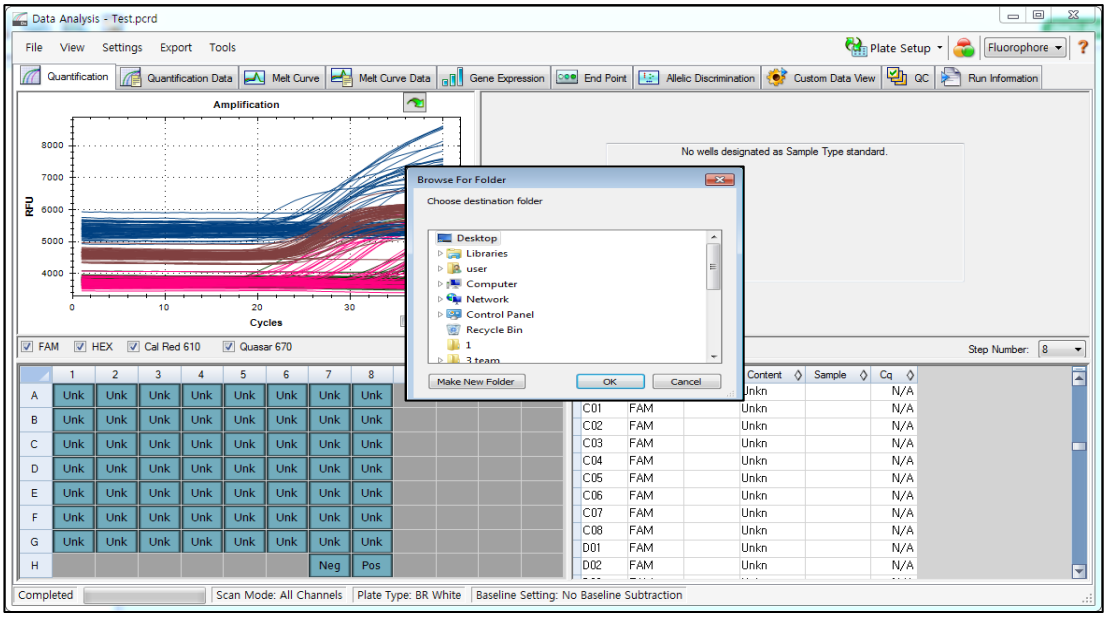


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

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

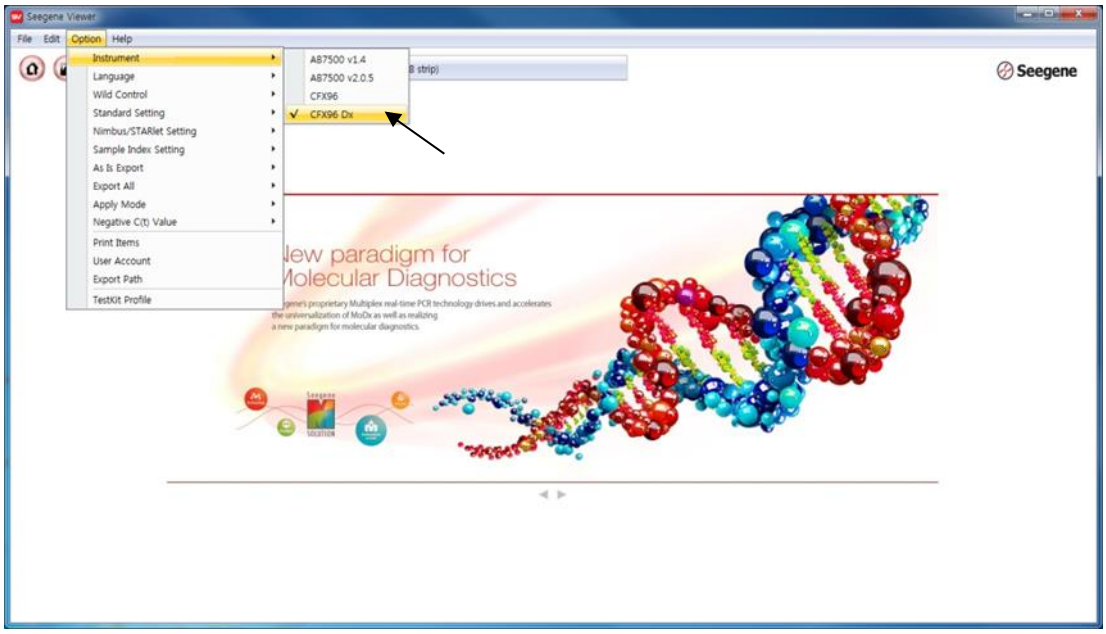


Fig. 13. **Seegene Viewer**

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

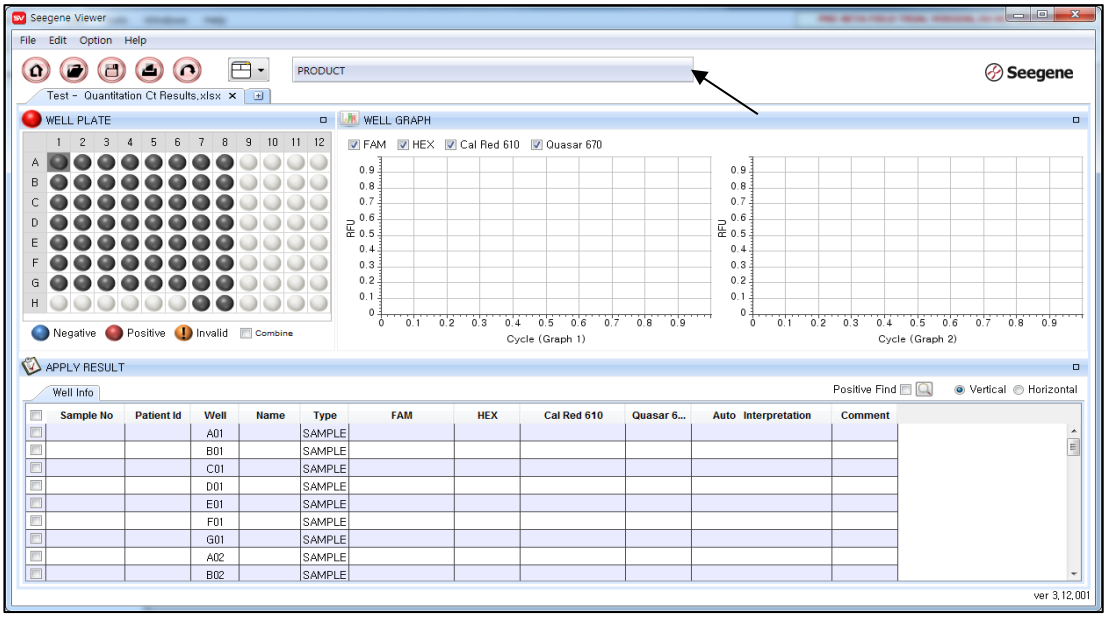


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

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

3) Check the result for each well.

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

4) Validity Criteria of Control Results

a. Valid Assay Run

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

Control	Seegene Viewer Result								
	FAM (Ct)		HEX (Ct)		Cal Red 610 (Ct)		Quasar670 (Ct)		Auto Interpretation
	UU	NG	MH	MG	UP	CT	TV	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

b. Invalid Assay Run

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

RESULTS

1. Analytes Information

Fluorophore	Analyte	
	Graph 1	Graph 2
FAM	*Ureaplasma urealyticum* (UU)	*Neisseria gonorrhoeae* (NG)
HEX	*Mycoplasma hominis* (MH)	*Mycoplasma genitalium* (MG)
Cal Red 610	*Ureaplasma parvum* (UP)	*Chlamydia trachomatis* (CT)
Quasar 670	*Trichomonas vaginalis* (TV)	Internal Control (IC)

2. Interpretation of Results

Analyte	Ct value	Result
Targets	≤ 40	Detected (+)
	N/A	Not detected (-)
IC	≤ 40	Detected (+)
	N/A	Not detected (-)

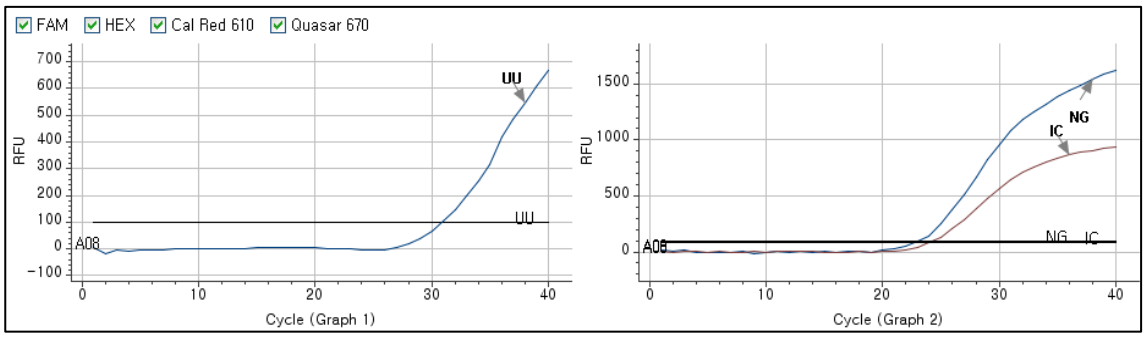
Target Result		IC Result	Interpretation
Graph 1	Graph 2		
+	-	+	**Target Nucleic acid, Detected**
-	+		
+	+		
+	-	-	**Target Nucleic acid, Detected*** - Additional STI targets that were not detected may be present.
-	+		
+	+		
-	-	+	**Target Nucleic acid, Not detected**
-	-	-	**Invalid**** - Negative IC signal suggests inadequate specimen collection, processing or presence of inhibitors. - Repeat the test from the nucleic acid extraction using another aliquot of the original specimen. - If the same result is shown in the re-extracted nucleic acid, please dilute (1/3~1/10) the specimen in saline solution and repeat the test from the extraction.

* Detection of Internal Control in the Quasar 670 channel is not required for positive results of target pathogens. High titer of another analyte may lead to reduced or absent Internal Control signal.

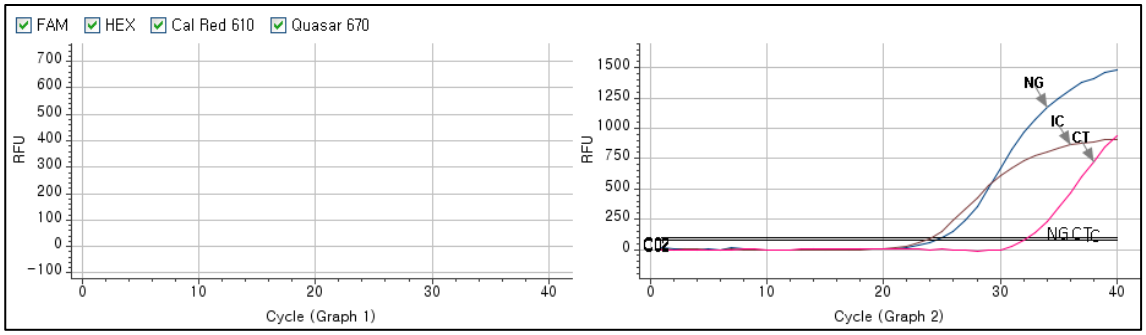
** If none of the signals including Internal Control is not observed, see TROUBLESHOOTINGS.

3. Application to Clinical Samples

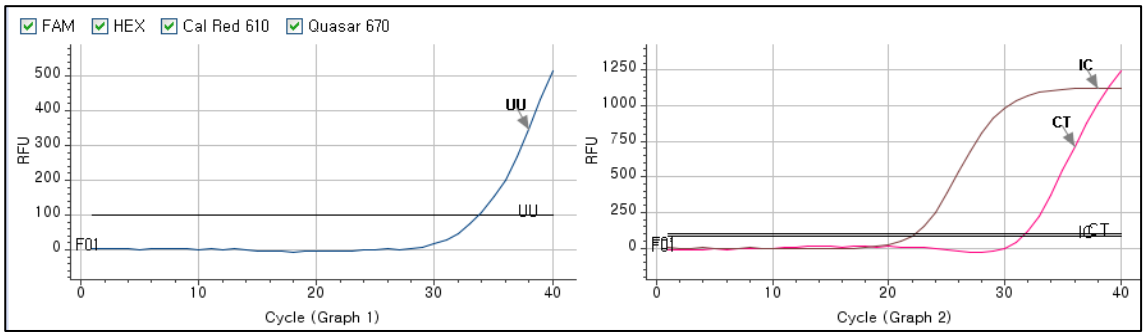
Sample 1



Sample 2



Sample 3



Sample	FAM				HEX				Cal Red 610				Quasar 670		Quasar 670		Auto Interpretation
	UU	Ct	NG	Ct	MH	Ct	MG	Ct	UP	Ct	CT	Ct	TV	Ct	IC	Ct	
1	+	30.96	+	23.19	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	+	23.97	UU,NG
2	-	N/A	+	25.09	-	N/A	-	N/A	-	N/A	+	32.42	-	N/A	+	23.76	NG,CT
3	+	33.76	-	N/A	-	N/A	-	N/A	-	N/A	+	31.80	-	N/A	+	21.82	UU,CT

TROUBLESHOOTINGS

Allplex™ STI Essential Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiration date of the test kit	Please check the storage conditions (See page 11) and the expiration date (refer to label) of the test kit and use a new kit if necessary.
	Nucleic acid extraction failure	If IC had been added to the specimen prior to extraction, absent signal of IC may indicate loss of nucleic acid during the extraction. Make sure that you use recommended extraction method. If due to inhibitors, re-extract the original specimen or the specimen may be diluted with saline solution 1/3~1/10 fold and then add ASTI IC to the diluted specimen. ASTI IC should be used only for urine specimen.
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.
	Presence of PCR Inhibitor	Please dilute the template nucleic acid (1/10~1/100) in RNase-free Water and repeat the test with the diluted nucleic acid. If specimen is still present, dilute the specimen (1/10~1/100) in Saline solution and repeat the test with the diluted specimen.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Centrifuge the PCR tube before run.

Allplex™ STI Essential Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative false positive or target signals observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.
Putative false negative or no signal observed in Positive Control	Error in specimen collection	Please check the specimen collection method, and re-collect the specimen.
	Incorrect storage of the specimen	Please re-collect the specimen and repeat the entire procedure. Ensure that the specimen is stored as recommended.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Presence of inhibitor	Please dilute the template nucleic acid (1/10~1/100) in RNase-free Water and repeat the test with the diluted nucleic acid. If specimen is still present, dilute the specimen (1/10~1/100) in saline solution and repeat the test with the diluted specimen.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture (Sensitivity is compromised with pre-composed premix). All reagents must be homogenized and spun down before use.

PERFORMANCE

1. Specificity

The high specificity of Allplex™ STI Essential Assay is ensured by the oligos designed specifically for the targets of interest and the set reaction conditions. Allplex™ STI Essential Assay was tested for cross-reactivity to 143 different pathogens, and PCR amplification and detection were only identified in the specified targets.

No.	Organism	Source	Isolate No.	Result †
1	*Chlamydia trachomatis*	ZMC	0804390	CT Detected
2	*Chlamydia trachomatis* (LGV I)	ATCC	VR-901BD	CT Detected
3	*Chlamydia trachomatis* (LGV II)	ATCC	VR-902BD	CT Detected
4	*Chlamydia trachomatis* (LGV III)	ATCC	VR-903D	CT Detected
5	*Chlamydia trachomatis* (serovar A)	ATCC	VR-571B	CT Detected
6	*Chlamydia trachomatis* (serovar B)	ATCC	VR-573	CT Detected
7	*Chlamydia trachomatis* (serovar Ba)	ATCC	VR-347	CT Detected
8	*Chlamydia trachomatis* (serovar C)	ATCC	VR-1477	CT Detected
9	*Chlamydia trachomatis* (serovar D)	ATCC	VR-885	CT Detected
10	*Chlamydia trachomatis* (serovar E)	ATCC	VR-348B	CT Detected
11	*Chlamydia trachomatis* (serovar F)	ATCC	VR-346	CT Detected
12	*Chlamydia trachomatis* (serovar G)	ATCC	VR-878	CT Detected
13	*Chlamydia trachomatis* (serovar H)	ATCC	VR-879	CT Detected
14	*Chlamydia trachomatis* (serovar I)	ATCC	VR-880	CT Detected
15	*Chlamydia trachomatis* (serovar J)	ATCC	VR-886	CT Detected
16	*Chlamydia trachomatis* (serovar K)	ATCC	VR-887	CT Detected
17	*Mycoplasma genitalium*	ATCC	49895	MG Detected
18	*Mycoplasma hominis*	ZMC	0804011	MH Detected
19	*Neisseria gonorrhoeae*	ZMC	0801482	NG Detected
20	*Neisseria gonorrhoeae*	ATCC	700825	NG Detected
21	*Neisseria gonorrhoeae*	NCTC	13798	NG Detected
22	*Neisseria gonorrhoeae*	NCTC	13800	NG Detected
23	*Neisseria gonorrhoeae*	NCTC	13817	NG Detected
24	*Trichomonas vaginalis*	ZMC	0801805	TV Detected

25	*Ureaplasma parvum*	ATCC	700970	UP Detected
26	*Ureaplasma urealyticum*	ATCC	33699	UU Detected
27	*Acinetobacter baumannii*	KCCM	35453	Not Detected
28	*Acinetobacter schindleri*	KCTC	12409	Not Detected
29	*Acinetobacter ursingii*	KCTC	12410	Not Detected
30	Adenovirus 40	ATCC	VR-931	Not Detected
31	*Arcanobacterium haemolyticum*	ATCC	BAA-1784	Not Detected
32	*Atopobium parvulum*	KCOM	1530	Not Detected
33	*Atopobium vaginae*	KCTC	15240	Not Detected
34	*Bacteroides caccae*	ATCC	43185	Not Detected
35	*Bacteroides fragilis*	KCTC	5013	Not Detected
36	*Bacteroides ovatus*	KCTC	5827	Not Detected
37	*Bacteroides vulgatus*	ATCC	8482	Not Detected
38	*Bacteroides xylanisolvens*	KCTC	15192	Not Detected
39	*Bifidobacterium adolescentis*	KCTC	3216	Not Detected
40	*Bifidobacterium longum*	KCTC	3421	Not Detected
41	*Bifidobacterium minimum*	KCTC	3273	Not Detected
42	*Campylobacter rectus*	KCTC	5636	Not Detected
43	*Candida albicans*	ATCC	10231D-5	Not Detected
44	*Candida dubliniensis*	KCTC	17427	Not Detected
45	*Candida glabrata*	KCCM	50044	Not Detected
46	*Candida krusei*	KCCM	11426	Not Detected
47	*Candida lusitaniae*	KCCM	50541	Not Detected
48	*Candida metapsilosis*	ATCC	96144D	Not Detected
49	*Candida orthopsilosis*	ATCC	96139	Not Detected
50	*Candida parapsilosis*	KCTC	7653	Not Detected
51	*Candida tropicalis*	ATCC	750	Not Detected
52	*Chlamydophila pneumoniae*	ATCC	VR-1310	Not Detected
53	*Chlamydophila psittaci*	Vircell	MBC013	Not Detected
54	*Clostridium difficile* (Toxin A+ / B+)	NCTC	11209	Not Detected
55	*Clostridium perfringens*	KCTC	3269	Not Detected
56	*Corynebacterium diphtheriae*	KCTC	3075	Not Detected
57	*Cytomegalovirus* (CMV)	NIBSC	09/162	Not Detected
58	*Enterococcus avium*	ATCC	14025	Not Detected

59	Enterovirus 70	ATCC	VR-836	Not Detected
60	Epstein Barr Virus	ATCC	VR-1492	Not Detected
61	*Escherichia coli*	ATCC	25922	Not Detected
62	*Fusobacterium necrophorum*	KCOM	1657	Not Detected
63	*Gardnerella vaginalis*	KCTC	5097	Not Detected
64	*Haemophilus ducreyi*	ATCC	700724D-5	Not Detected
65	*Haemophilus influenzae*	KCCM	42099	Not Detected
66	*Helicobacter pylori*	ZMC	0804383	Not Detected
67	Hepatitis A virus (HAV)	ATCC	VR-1541	Not Detected
68	Hepatitis B virus (HBV)	ATCC	VR-3232SD	Not Detected
69	Hepatitis C virus (HCV)	ATCC	VR-3233SD	Not Detected
70	Human herpesvirus 1	ATCC	VR-260	Not Detected
71	Human herpesvirus 2	ATCC	VR-734	Not Detected
72	Human herpesvirus 3	ATCC	VR-1367	Not Detected
73	Human Papilloma Virus 16	KCLB	30035	Not Detected
74	Human Papilloma Virus 16	KCLB	21550	Not Detected
75	Human Papilloma Virus 18	KCLB	10002	Not Detected
76	*Lactobacillus acidophilus*	KCTC	3140	Not Detected
77	*Lactobacillus amylovorus*	KCTC	3179	Not Detected
78	*Lactobacillus brevis*	KCTC	3498	Not Detected
79	*Lactobacillus casei*	KCTC	3260	Not Detected
80	*Lactobacillus crispatus*	KCTC	5054	Not Detected
81	*Lactobacillus delbrueckii subsp. Delbrueckii*	KCTC	13730	Not Detected
82	*Lactobacillus fermentum*	KCTC	3112	Not Detected
83	*Lactobacillus gallinarum*	KCTC	5048	Not Detected
84	*Lactobacillus gasseri*	KCTC	3163	Not Detected
85	*Lactobacillus helveticus*	KCTC	15060	Not Detected
86	*Lactobacillus iners*	CCARM	123	Not Detected
87	*Lactobacillus intestinalis*	KCTC	5052	Not Detected
88	*Lactobacillus jensenii*	KCTC	5194	Not Detected
89	*Lactobacillus johnsonii*	KCTC	3801	Not Detected
90	*Lactobacillus kefiranofaciens*	KCTC	5075	Not Detected
91	*Lactobacillus oris*	KCCM	40993	Not Detected
92	*Lactobacillus parabuchneri*	KCTC	3503	Not Detected

93	*Lactobacillus pentosus*	KCTC	3120	Not Detected
94	*Lactobacillus plantarum*	ATCC	700934	Not Detected
95	*Lactobacillus reuteri*	KCTC	3679	Not Detected
96	*Lactobacillus rhamnosus*	KCCM	32405	Not Detected
97	*Lactobacillus salivarius* subsp. salicinius	KCTC	3600	Not Detected
98	*Lactobacillus sanfrancisensis*	KACC	12431	Not Detected
99	*Lactobacillus ultunensis*	KCTC	5857	Not Detected
100	*Lactobacillus vaginalis*	KCTC	3515	Not Detected
101	*Mobiluncus curtisii*	ATCC	35241	Not Detected
102	*Mobiluncus mulieris*	ATCC	35243	Not Detected
103	*Moraxella catarrhalis*	KCCM	42706	Not Detected
104	*Mycoplasma arginini*	ATCC	23838	Not Detected
105	*Mycoplasma felis* Cole et al.	ATCC	23391	Not Detected
106	*Mycoplasma iowae* Jordan et al.	ATCC	33552	Not Detected
107	*Mycoplasma leonicaptivi* Hill	ATCC	49890	Not Detected
108	*Mycoplasma pneumonia*	ATCC	15531	Not Detected
109	*Mycoplasma pulmonis*	ATCC	19612	Not Detected
110	*Mycoplasma spumans*	ATCC	19526	Not Detected
111	*Neisseria cinerea*	ATCC	14685	Not Detected
112	*Neisseria elongata*	ZMC	801510	Not Detected
113	*Neisseria flavescens*	CCARM	9264	Not Detected
114	*Neisseria flavescens*	ATCC	13120	Not Detected
115	*Neisseria lactamica*	ATCC	23970	Not Detected
116	*Neisseria lactamica*	ZMC	801752	Not Detected
117	*Neisseria meningitidis*	ATCC	700532D	Not Detected
118	*Neisseria meningitidis*	KCCM	41562	Not Detected
119	*Neisseria mucosa*	ATCC	19696	Not Detected
120	*Neisseria mucosa*	KCCM	11703	Not Detected
121	*Neisseria perflava*	ATCC	14799D-5	Not Detected
122	*Neisseria polysaccharea*	ZMC	804030	Not Detected
123	*Neisseria sicca*	ATCC	29256	Not Detected
124	*Neisseria sicca*	ZMC	801754	Not Detected
125	*Neisseria subflava*	ATCC	49275	Not Detected
126	*Neisseria subflava*	ZMC	804298	Not Detected

127	Norovirus GII 17	ATCC	VR-3200SD	Not Detected
128	*Peptostreptococcus micros*	KCTC	15021	Not Detected
129	*Prevotella bivia*	KCTC	5454	Not Detected
130	*Prevotella buccalis*	KCTC	5496	Not Detected
131	*Prevotella disiens*	KCTC	5499	Not Detected
132	*Prevotella intermedia*	KCTC	5692	Not Detected
133	*Prevotella melaninogenica*	KCTC	5457	Not Detected
134	*Pseudomonas aeruginosa*	KCOM	1182	Not Detected
135	*Saccharomyces cerevisiae*	KCCM	50511	Not Detected
136	*Salmonella enteritidis*	CCARM	8570	Not Detected
137	*Salmonella typhimurium*	CCARM	270	Not Detected
138	*Staphylococcus aureus*	KCOM	1335	Not Detected
139	*Streptococcus agalactiae*	ATCC	BAA-611D-5	Not Detected
140	*Streptococcus pneumoniae*	ATCC	BAA-255D	Not Detected
141	*Treponema pallidum*	ATCC	BAA-2642SD	Not Detected
142	*Trichomonas tenax*	ATCC	30207	Not Detected
143	*Vibrio parahaemolyticus*	KCTC	2471	Not Detected

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

※ ATCC: American Type Culture Collection
CCARM: Culture Collection of Antimicrobial Resistant Microbes
KACC: Korean Agricultural Culture Collection
KCCM: Korean Culture Center of Microorganisms
KCLB: Korean Cell Line Bank
KCOM: Korea Collection for Oral Microbiology
KCTC: Korean Collection for Type Culture
NCTC: National Collection of Type Cultures
NIBSC: National Institute for Biological Standards and Control
Vircell: Vircell microbiologists
ZMC: ZeptoMetrix Corporation

2. Sensitivity

The sensitivity is defined as the lowest concentration of organism that can be consistently detected (≥ 95% of positive results among all tested sample.

The sensitivity of Allplex™ STI Essential Assay was estimated using probit analysis with serial dilutions of quantified standard organisms. Furthermore, the sensitivity of Allplex™ STI Essential Assay was determined using nucleic acids extracted and quantified as genomic copies/reaction. The claimed detection limit of targets of Allplex™ STI Essential Assay is as shown in the table below.

Organism	Standard organism		Genomic DNA
	Source	Detection limit	Detection limit (genomic copies/reaction)
Ureaplasma urealiticum	ATCC 33699	3.00 x 10^1 CCU/ml	10^3
Neisseria gonorrhoeae	ZeptoMetrix 0801482	6.36 x 10^0 CFU/ml	10^1
Mycoplasma hominis	ZeptoMetrix 0804011	2.69 x 10^3CCU/ml	10^2
Mycoplasma genitalium	ATCC 49895	2.70 x 10^2 CFU/ml	5 x 10^1
Ureaplasma parvum	ATCC 700970	2.69 x 10^2 CCU/ml	10^5
Chlamydia trachomatis	ZeptoMetrix 0804390	6.73 x 10^0 IFU/ml	10^1
Trichomonas vaginalis	ZeptoMetrix 0801805	4.91 x 10^1 cells/ml	10^1

3. Reproducibility

The reproducibility panel of 21 simulated analytes was prepared that included High negative (0.1 X LoD), Low positive (1X LoD) and Moderate positive (3X LoD) samples. At each testing site, the panel was tested for five days, two runs per day by two different operators and triplicate of each panel per run from one extraction. It was tested with a single lot of Allplex™ STI Essential Assay at three different sites and three lots at one in-house site. The positive rates were observed for each analytes for reproducibility study: 100.00% for Moderate positive samples, ≥100.00% for Low positive samples and ≥0.00% for High negative samples.

The reproducibility of Allplex™ STI Essential Assay was evaluated between sites, product lots and experimenters. The results were satisfied with the criteria, thus confirming the reproducible performances of Allplex™ STI Essential Assay.

4. Repeatability

The repeatability panel of 21 simulated analytes were prepared that included High negative (0.1X LoD), Low positive (1X LoD) and Moderate positive (3X LoD) samples. It was tested at in house (Seegene) 3 times for 20 days, two runs per day (Total N = 120 tests). The positive rates were observed for each analyte for repeatability study: 100.00% for Moderate positive samples,

100.00% for Low positive samples and ≥2.50% for High negative samples. The results were satisfied with the criteria, thus confirming the repeatable performances of Allplex™ STI Essential Assay.

5. Interfering substances

This test was conducted using interfering substances composed of 20 substances in order to confirm the performance of the Allplex™ STI Essential Assay in the presence of potential interfering substances. There was no effect on the result by adding the substances: non-specific detection or inhibition on target amplification. Based on the results, 20 interfering substances had no effect on Allplex™ STI Essential Assay results.

No.	Interfering substances	Concentration
1	Metronidazole	701 µmol/L
2	Amoxicillin	206 µmol/L
3	Bilirubin	257 µmol/L
4	Hemoglobin human	200 g/L
5	Progesterone	20 ng/ml
6	Beta Estradiol	4.41 nmol/L
7	Acetylsalicylic Acid (aspirin)	3.62 mmol/L
8	Glucose	12.2 mmol/L
9	Albumin from human serum	52 g/L
10	Mucin	3 mg/mL
11	Testosterone	41.6 nmol/L
12	Luteinizing hormone (LH)	70 IU/L
13	Follicle Stimulating Hormone (FSH)	100 IU/L
14	Cortisol	828 nmol/L
15	Fructose	1000 µmol/L
16	Suppositories/hemorrhoidal treatment	5% w/v
17	Feces	1% w/v
18	Cough suppressant	5% v/v
19	Toothpaste	5% v/v
20	Mouthwash	5% v/v

6. Clinical study

A total of 2020 clinical specimens were tested with Allplex™ STI Essential Assay and reference assay.

The agreements between Allplex™ STI Essential Assay (V3.0) and reference assay, with reflection of sequencing confirmation, were 99.60%, 99.75%, 99.55%, 99.60%, 99.55%, 99.85% and 99.95% for detection of UU, NG, MH, MG, UP, CT, and TV, respectively.

The clinical validity of Allplex™ STI Essential Assay (V3.0) has been proven in diagnosing seven STI analytes, as the results satisfy the success criteria.

Analyte	PPA (compared to reference assay)			NPA (compared to reference assay)			Agreement		
	TP/ (TP+FN)	%[a)]	95% CI[c)]	TN/ (TN+FP)	%[b)]	95% CI[c)]	(TP+TN)/ Total	%[d)]	95% CI[c)]
Ureaplasma urealiticum (UU)	434/436	99.54	98.35 ~ 99.94	1578 /1584	99.62	99.18 ~ 99.86	2012 /2020	99.60	99.22 ~ 99.83
Neisseria gonorrhoeae (NG)	188/189	99.47	97.09 ~ 99.99	1827 /1831	99.78	99.44 ~ 99.94	2015 /2020	99.75	99.42 ~ 99.92
Mycoplasma hominis (MH)	344/346	99.42	97.93 ~ 99.93	1667 /1674	99.58	99.14 ~ 99.83	2011 /2020	99.55	99.16 ~ 99.80
Mycoplasma genitalium (MG)	263/263	100.00	98.61 ~ 100.00	1749 /1757	99.54	99.11 ~ 99.80	2012 /2020	99.60	99.22 ~ 99.83
Ureaplasma parvum (UP)	519/528	98.30	96.79 ~ 99.22	1492 /1492	100.00	99.75 ~ 100.00	2011 /2020	99.55	99.16 ~ 99.80
Chlamydia trachomatis (CT)	261/262	99.62	97.89 ~ 99.99	1756 /1758	99.89	99.59 ~ 99.99	2017 /2020	99.85	99.57 ~ 99.97
Trichomonas vaginalis (TV)	168/169	99.41	96.75 ~ 99.99	1851 /1851	100.00	99.80 ~ 100.00	2019 /2020	99.95	99.72 ~ 100.00

a) PPA (Positive percent agreement) (%): 100 X TP/(TP+FN)

b) NPA (Negative percent agreement) (%): 100 X TN/(FP+TN)

c) The two-sided 95% confidence intervals were calculated.

d) Agreement (%): 100 X (TP+TN)/(TP+TN+FP+FN)

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KEY TO SYMBOLS

Key to symbols used in the manual and labels.

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use-by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
PREMIX	PCR Master Mix or Detection Mix
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	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

Symbol	Explanation
rxns	Reaction barcode for automated extraction system

ORDERING INFORMATION

Cat. No.	Product	Size
Allplex™ series		
SD10245Z	Allplex™ STI Essential Assay	25 rxns
SD9801Y	Allplex™ STI Essential Assay	50 rxns
SD9801X	Allplex™ STI Essential Assay	100 rxns
SD10318Z	Allplex™ STI Essential Assay Q(MH,UU)	25 rxns
SD10201Y	Allplex™ STI Essential Assay Q(MH,UU)	50 rxns
SD10202X	Allplex™ STI Essential Assay Q(MH,UU)	100 rxns
SD10177Z	Allplex™ Genital ulcer Assay	25 rxns
SD9802Y	Allplex™ Genital ulcer Assay	50 rxns
SD9802X	Allplex™ Genital ulcer Assay	100 rxns
SD10178Z	Allplex™ Candidiasis Assay	25 rxns
SD9803Y	Allplex™ Candidiasis Assay	50 rxns
SD9803X	Allplex™ Candidiasis Assay	100 rxns
SD9804X	AllplexTM Bacterial Vaginosis Assay	100 rxns
SD10320Z	AllplexTM Bacterial Vaginosis plus Assay	25 rxns
SD10159X	Allplex™ Bacterial Vaginosis *plus* Assay	100 rxns
SD10317Z	Allplex™ CT/NG/MG/TV Assay	25 rxns
SD9400Y	Allplex™ CT/NG/MG/TV Assay	50 rxns
SD9400X	Allplex™ CT/NG/MG/TV Assay	100 rxns
SD10319Z	Allplex™ MG & AziR Assay	25 rxns
SD10169Y	Allplex™ MG & AziR Assay	50 rxns
SD10170X	Allplex™ MG & AziR Assay	100 rxns
SD10232Z	Allplex™ MG & MoxiR Assay	25 rxns
SD10233Y	Allplex™ MG & MoxiR Assay	50 rxns
SD10234X	Allplex™ MG & MoxiR Assay	100 rxns
SD10368Z	Allplex™ NG & DR Assay	25 rxns
SD10367X	Allplex™ NG & DR Assay	100 rxns

Anyplex™ series		
SD7700Y	Anyplex™ II STI-7 Detection (V1.1)	50 rxns
SD7700X	Anyplex™ II STI-7 Detection (V1.1)	100 rxns
SD7500Y	Anyplex™ II STI-5 Detection	50 rxns
SD7500X	Anyplex™ II STI-5 Detection	100 rxns
SD10323Z	Anyplex™ II STI-7e Detection	25 rxns
SD7701Y	Anyplex™ II STI-7e Detection	50 rxns
SD7701X	Anyplex™ II STI-7e Detection	100 rxns
SD7200Y	Anyplex™ CT/NG Real-time Detection (V3.1)	50 rxns*

* In case of SmartCycler® II System, total rxn number is reduced to 40 rxn from 50 rxn. (50 rxns→40 rxns)

Seeplex® series		
HS6200Y	Seeplex® HSV2 ACE Detection	50 rxns
SD6401Y	Seeplex® STD4D ACE Detection (V2.0)	50 rxns
SD6600Y	Seeplex® STD6 ACE Detection (V2.0)	50 rxns
SD6511Y	Seeplex® STI Master Panel 1 (V2.0)	50 rxns

Accessory products		
SG1701	Ribo_spin vRD (Viral RNA/DNA Extraction Kit)	50 preps

Automated extraction Systems		
65415-02	Microlab NIMBUS IVD	EA
173000-075	Microlab STARlet IVD	EA
65415-03	Seegene NIMBUS	EA
67930-03	Seegene STARlet	EA
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384T / 1box
EX00013C	STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384T / 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
SG72100	AIOS	EA