Allplex™

STI Essential Assay

(Cat. No.SD9801Y)

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)





For in vitro diagnostic use only



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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 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 <u>DNA</u> samples at ≤ -20°C 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.
- Always wear disposable gloves in each area and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Dedicate supplies and equipment to separate working areas and do not move them 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°C. Please refer to label for final expiry date.
- 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 detection 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).

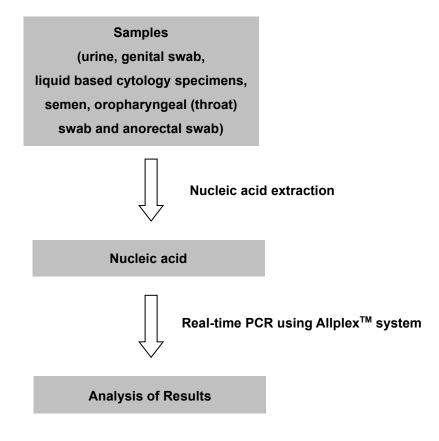
In Allplex[™] STI Essential Assay, an endogenous human gene is used as Internal Control (IC) for monitoring the whole process from sample collection to nucleic acid extraction as well as to check for any possible PCR inhibition. PCR efficiency may be reduced by inhibitors that may be present in clinical specimens. However, due to the inconsistencies in the amount 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 a 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 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 50 reactions.

Order information (REF	SD9801Y)
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Allplex [™] STI Essential Assay							
Symbol	Contents	Volume	Description				
PRIMER	PRIMER 4X STI-EA MOM		MuDT Oligo Mix (MOM): - Amplification and detection reagent				
PREMIX	EM1	- DNA polymerase 250 μL - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs					
CONTROL +	STI-EA PC	25 µL	Positive Control (PC) - Mixture of pathogen clones				
CONTROL	ASTI IC	500 µL	Internal Control (IC) for urine and anorectal swab specimen				
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade				
Í		ι	Jser manual				



STORAGE AND HANDLING

All components of the AllplexTM STI Essential Assay should be stored at \leq -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
- Nucleic acid extraction kit (see Nucleic Acid Extraction)
- Ice Maker
- Desktop centrifuge
- Mini plate spiner Centrifuge
- Vortex mixer
- CFX96TM Real-time PCR Detection system (Bio-Rad)
- CFX96TM 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)
- 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.

<u>Urine specimen</u> <u>Genital swab specimen</u> <u>Liquid based cytology specimen</u> <u>Semen</u> <u>Oropharyngeal (throat) swab specimen</u> <u>Anorectal swab specimen</u>

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

<u>Urine specimen</u>

- 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 specimen, 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.

<u>Semen</u>

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

C = -		Storage & Tra	nsport	Nete
Spe	ecimen	Temp.	Duration*	Note
Urine	specimen	2~8°C	1 week	
Genital swab specimen		2~8°C	1 week	
Liquid based	ThinPrep [®] medium CellPreserv	2~8℃ ^{**} & Room Temperature**	90 days	- Performance may be affected by prolonged storage of
cytology	SurePath [™] medium	2~8℃	2 weeks	specimens. - Specimens should also adhere to local and national
Semen		2~8℃	1 week	instructions for transport of
	ngeal (throat) wab	2~8℃	3 days	pathogenic material.
Apora	ectal swab	2~8℃	2 days	
Anore	ระเล่า รพสม	-20 ℃	1 month	

B. Specimen Storage & Transport

* 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 (NucliSENS[®] easyMAG[®] and SEEPREP32)

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 $(19 \sim 25 ^{\circ} C)$.
- 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, 2.D) by thoroughly vortexing.

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

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

• Follow the manufacturer's protocol.

<u>Semen</u>

- Equilibrate semen for 30 min in darkness until liquefaction in the room temperature (19~25°C).
- Dilute three times with Saline solution at recommended volume (See Recommended Vol. Of 2.C, 2.D-1) 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 the each specimen before the nucleic acid extraction.

C. 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 and anorectal swab specimen resuspend the pellet with 190 μ L of saline solution and add 10 μ L of ASTI IC.



D. 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.

D-1. NucliSENS® easyMAG®

• Proceed the extraction process using <u>'generic protocol'</u>.

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

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

Note: SurePath[™] have not been validated with NucliSENS[®] easyMAG[®].

D-2. SEEPREP32

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	Seegene	EX00017P	Specimen: 200 µL*
Туре)	Seegene	LX00017F	Elution: 100 µL
STARMag 96 ProPrep C (Tube	Sociano	EX00017T	Specimen: 200 µL*
Туре)	Seegene	EXUUUTIT	Elution: 100 µL

• Proceed the extraction process using <u>'Pro-Protocol A'</u>.

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

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



Note: Semen have not been validated with SEEPREP32.

E. Summary

Extraction Method	Applicated sampling device
NucliSENS [®] easyMAG [®]	ENAT, UTM, ThinPrep [®] , CellPreserv, Urine, Semen,
system	Oropharyngeal (throat) swab, Anorectal swab
QIAamp [®] DSP DNA Mini Kit	ENAT, UTM, ThinPrep [®] , CellPreserv, SurePath ^{™ 1} , Urine,
QIAamp [®] DNA Mini Kit	Semen, Oropharyngeal (throat) swab, Anorectal swab
Ribo_spin vRD	ENAT, UTM, ThinPrep [®] , CellPreserv, Urine, Semen,
(Viral RNA/DNA Extraction Kit)	Oropharyngeal (throat) swab, Anorectal swab
SEEPREP32	ENAT, UTM, ThinPrep [®] , CellPreserv, Urine

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

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: Briefly centrifuge the reagent tubes to remove drops from inside of the cap.

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 briefly centrifuge.
- **C.** Aliquot 15 μ L of the PCR Mastermix into PCR tubes.



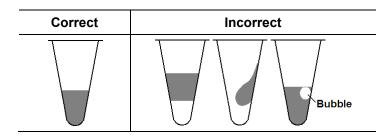
D. Add 5 μ L of each sample's nucleic acids into the tube containing the PCR Mastermix.

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

E. Close the cap, and briefly centrifuge 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 μ L of RNase-free Water instead of sample's nucleic acid.

Note: For **Positive Control (PC)**, use 5 μ L 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.



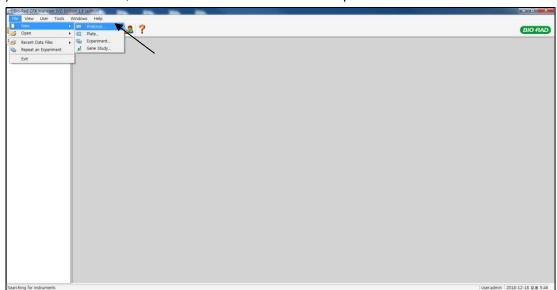
REAL-TIME PCR INSTRUMENT SET UP AND RESULTS ANALYSIS

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



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

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

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

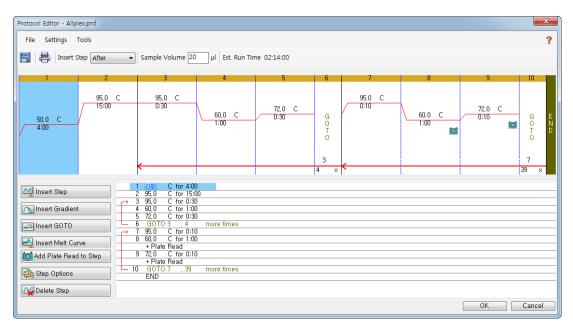


Fig. 2. Protocol Editor

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



- Experiment Setup × Options Mart Protocol 💷 Plate 🕩 Start Run Create New... Express Load • Select Existing... Selected Protocol Edit Selected,, Allplex, prcl Preview Est, Run Time: 02:14:00 (96 Wells-All Channels) Sample Volume: 20u 95,0 C 15:00 95,0 C 0:30 95,0 C 0:10 72,0 C 0:30 72,0 C 0:10 60,0 C 1:00 60,0 C 1:00 G O T O G O T O Ó 50,0 C 4:00 Ó Next >>
- 4) Click OK and save the protocol to open the Experiment Setup window.

Fig. 3. Experiment Setup: Protocol

B. Plate Setup

· Pr	ns rotocol 💷	Plate.	Start Bun									
_			otart Hun									
	Create New,								Express Loa		Less to she	
	elect Existing ted Plate		•						QuickPlate_9	I6 WEIISLAII C	nanneis, pito	
		s_All Channel	ls.pltd								Edit Se	lected
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Fluoro	phores:	FAM, H	IEX, Texas R	ed, Cy5, Quas	ar 705			Plate Typ	e: BR Clear		Scan Mode:	All Channe
	1	2	3	4	5	6	7	8	9	10	11	12
А	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
в	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
С	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
υ	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
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G	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
н	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk

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.

	2 3	4 5 Select Fluorophores	6 7	8 9 10	11 12	🤖 Select Flu	orophores
3		Channel	Fluorophore	Selected Colo		Sample Type	
;			FAM			Load	Target Name <pre></pre>
)		2	HEX TET			Load	Sample Name
			Cal Gold 540 VIC				<none></none>
		3	ROX			Load	Replicate #
ì			Texas Red Cal Red 610				i' ate Series
1		4	Cy5 Quasar 670			000	nt Settings
		5	Quasar 705		_		Replicate #
				OK	Cancel	Clea	ar Wells

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).

Plate Ed	ditor - Test.p	td									press Local				×
File	Settings	Tools													
			• 🔯	Scan Mode 🖟	All Channels	•	al well a	Groups 🕴	•				<u>e</u>	Plate Loading	Guide
	Plate		· · ·	on mine	X										
	Units	ber Conventio	on 🖡	BR Clear	Unk	6 Unk	7 Unk	8 Unk	9	10	11	12	Select Flu	orophores	
A	HEX Cal Red 610 Quasar 670	HEX Cal Red 610 Quasar 670	HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670					Sample Type	Unknown	-
в	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					Load FAM HEX	Target Name <none> <none></none></none>	•
с	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					Cal Red 610	<none></none>	-
D	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					Load	Sample Name <none> Replicate #</none>	•
Е	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unic FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unic FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670						1 ate Series ent Settings	÷
F	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unic FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670						Replicate # ar Wells	
G	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670							
н							Neg FAM HEX Cal Red 610 Quasar 670	Pos FAM HEX Cal Red 610 Quasar 670							
Pla	ate Type : Bl	R White											OK	Cance	

Fig. 6. Plate Setup

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

· ·	ns rotocol 💷	Plate 🕠	Start Run									
S	Create New, elect Existins								Express Loa	ad		
	ted Plate											
Test, p Previe											Edit Se	ected
	phores:	FAM, H	IEX, Cal Red	610, Quasar 6	70			Plate Type	e: BR White		Scan Mode:	All Channel
	1	2	3	4	5	6	7	8	9	10	11	12
А	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
в	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
C	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
υ	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
G	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
н							Neg	Pos				

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.

Experiment Setup			
Options			
Protocol III Plate III Start Run			
Run Information			
Protocol : Allplex.prcl			
Plate : Test.pltd			
Notes :	^ ~		
Scan Mode : All Channels			
Start Run on Selected Block(s)			
Block Name △ Type ✓ BR100160 "96FX"	Run Status	Sample Volume 20	Protocol ID
Select All Blocks Flash Block Indicator Z Open Lid Cla	ise Lid		
			Start Run
			<pre>></pre>

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.

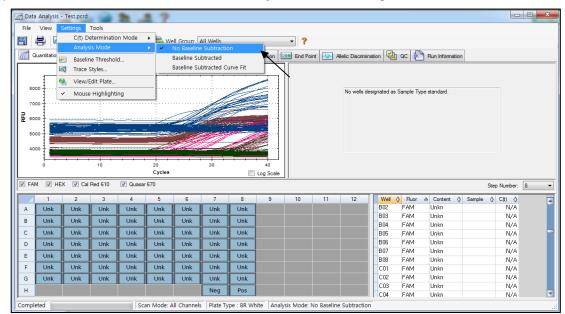
2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).



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

- Data Analysis Test.pcrd View Settings File Tools 📙 🛃 🔯 🔃 👫 View/Edit Plate... 🚠 Well Group: All Wells - ? Quantital 🕼 Quantitation Data 🔊 Met Curve 🔄 Met Curve Data 🔐 Gene Expression 🔤 End Point 🔛 Alleic Discrimination 🖓 QC 🖹 Run Information 300 250 No wells designated as Sample Type standard 2000 P 1500 1000 20 Cycles I Lon Sc FAM V HEX V Cal Red 610 Quasar 670 Step Number: 8 10 ♦ C(t) ♦ N/A Well E08 ♦ Fluor ▲ Content Cal Red 6 Unkn \diamond Unk Unk Unk Unk Unk Unk Unk А Unk Cal Red 61(Unkn 32.55 F01 в Unk Unk Unk Unk Unk Unk Unk Unk F02 Cal Red 61(Unkn 32.80 F03 F04 F05 N/A N/A 22,70 Cal Red 61(Unkn Cal Red 61(Unkn с Unk Unk Unk Unk Unk Unk Unk Unk D Unk Unk Unk Unk Unk Unk Unk Unk Cal Red 6 Unkn Е Unk Unk Unk Unk Unk Unk Unk Unk Cal Red 61(Unkn Cal Red 61(Unkn N/A 26.24 F06 F07 F Unk Unk Unk Unk Unk Unk Unk Unk G Unk Unk Unk Unk Unk Unk Unk Unk F08 Cal Red 61(Unkn N/A Cal Red 61(Unkn Cal Red 61(Unkn G01 31.68 н Pos Neg G02 29.69 Scan Mode: All Channels Plate Type : BR White Analysis Mode: Baseline Subtracted Curve Fit Completed
- 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.

File		- Test.pcrd Settings	Tools	2	1.7													
	8 🛛		🕄 Repor	ts rt Fluorophe	ore Calibrat	ion	I Wells			- ?								
🧖 Qı	uantitation	🧖 Qu		ce Plate	ore calibrat	1011	e Data 🔒	Gene E	xpression	nd Poi	nt 🔛 A	Ilelic Discrimin	nation	QC 🛃	Run Informat	tion		
			Expor	t All Data S	heets to Ex	cel												
			Seege	ene Export														
800	» †							A CONTRACTOR OF				No wells de	esignated as	Sample Ty	pe standard.			
700																		
600	1				-	-71		- FEE										
600		=				<u></u>												
500	00		<u> </u>			Stant -												
400		_			10	-//												
	~ 		_															
	0		10		20		30		40									
	•		10					_										
1					Cycles				Log Scale									
FAN	v I I HE	X 🔽 Cal		🔽 Quasar	Cycles												Step Number:	8
FAN	1 💌 HE	X <table-cell> Cal</table-cell>		Quasar	Cycles	6	7	8		10	11	12		> Fluor	△ Content	♦ Sample	♦ C(t) ♦	8
	1 Vnk		Red 610		Cycles 670	6 Unk			Log Scale	10	11	12	B04	FAM	Unkn	♦ Sample	♦ C(t) ♦	8
A	1	2	Red 610	4	Cycles 670 5		7	8	Log Scale	10	11	12	B04 B05	FAM FAM	Unkn Unkn	♦ Sample	♦ Ctt) ♦ N/A N/A	8
A B	1 Unk	2 Unk	Red 610 3 Unk	4 Unk	Cycles 670 5 Unk	Unk	7 Unk	8 Unk	Log Scale	10	11	12	B04 B05 B06	FAM FAM FAM	Unkn	♦ Sample		8
A B C	1 Unk Unk Unk	2 Unk Unk Unk	Red 610 3 Unk Unk Unk	4 Unk Unk Unk	Cycles 670 5 Unk Unk Unk	Unk Unk Unk	7 Unk Unk Unk	8 Unk Unk Unk	Log Scale	10	11	12	B04 B05	FAM FAM	Unkn Unkn Unkn	♦ Sample	♦ Ctt) ♦ N/A N/A	8
A B C D	1 Unk Unk Unk Unk	2 Unk Unk Unk Unk	Red 610 3 Unk Unk Unk Unk	4 Unk Unk Unk Unk	Cycles 670 5 Unk Unk Unk Unk	Unk Unk Unk Unk	7 Unk Unk Unk Unk	8 Unk Unk Unk Unk	Log Scale	10	11	12	B04 B05 B06 B07	FAM FAM FAM FAM	Unkn Unkn Unkn Unkn	♦ Sample		8
A B D E	1 Unk Unk Unk Unk Unk	2 Unk Unk Unk Unk Unk	Red 610 3 Unk Unk Unk Unk Unk	4 Unk Unk Unk Unk Unk	Cycles 670 5 Unk Unk Unk Unk Unk	Unk Unk Unk Unk Unk	7 Unk Unk Unk Unk Unk	8 Unk Unk Unk Unk Unk	Log Scale	10	11	12	804 805 806 807 808	FAM FAM FAM FAM FAM FAM	Unkn Unkn Unkn Unkn Unkn	♦ Sample		8
A B D E	1 Unk Unk Unk Unk	2 Unk Unk Unk Unk	Red 610 3 Unk Unk Unk Unk	4 Unk Unk Unk Unk	Cycles 670 5 Unk Unk Unk Unk	Unk Unk Unk Unk	7 Unk Unk Unk Unk	8 Unk Unk Unk Unk	Log Scale	10	11	12	B04 B05 B06 B07 B08 C01 C02 C02 C03	FAM FAM FAM FAM FAM FAM FAM	Unkn Unkn Unkn Unkn Unkn Unkn Unkn Unkn	♦ Sample		8
A B C D E F	1 Unk Unk Unk Unk Unk	2 Unk Unk Unk Unk Unk	Red 610 3 Unk Unk Unk Unk Unk	4 Unk Unk Unk Unk Unk	Cycles 670 5 Unk Unk Unk Unk Unk	Unk Unk Unk Unk Unk	7 Unk Unk Unk Unk Unk	8 Unk Unk Unk Unk Unk	Log Scale	10	11	12	B04 B05 B06 B07 B08 C01 C02 C03 C03 C04	FAM FAM FAM FAM FAM FAM FAM FAM	Unkn Unkn Unkn Unkn Unkn Unkn Unkn Unkn	Sample	 ♦ C(t) ♦ N/A N/A N/A N/A N/A N/A N/A N/A N/A 	8
A B C	1 Unk Unk Unk Unk Unk	2 Unk Unk Unk Unk Unk Unk	Red 610 3 Unk Unk Unk Unk Unk	4 Unk Unk Unk Unk Unk Unk	Cycles 670 5 Unk Unk Unk Unk Unk Unk	Unk Unk Unk Unk Unk Unk	7 Unk Unk Unk Unk Unk Unk	8 Unk Unk Unk Unk Unk Unk	Log Scale	10	11	12	B04 B05 B06 B07 B08 C01 C02 C02 C03	FAM FAM FAM FAM FAM FAM FAM	Unkn Unkn Unkn Unkn Unkn Unkn Unkn Unkn	Sample		8

Fig. 11. Seegene Export

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

		Test.pcrd Settings	Tools	2	1 7											
	8 🛛			Edit Plate	. 📸 We	ll Group: 🖌	All Wells		- ?							
<u></u>	antitation	Guar	ntitation Data	Me	t Curve 🚽	Melt Curv	re Data	Gene Expression	🖭 End Point 🔛 A	lelic Discriminat	ion 🛛	🖢 ac 훋	Run Information	r		
				Amp	lification											
800	° †						Bro	wse For Folder	1	-×	Ted :	as Sample Typ	e standard.			
700 2 600 500 400		X Cal	10 Red 610	Quasar	20 Cycles			hoose destination folder Desktop Computer Second		× E				Step 1	Number: [8 -
	1	2	3	4	5	6	-	Make New Folder	ок	Cancel	Vell	O Fluor	△ Content ()	Sample 👌 (C(t) ()	
A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Опк		_	04	FAM	Unkn		N/A	
в	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			B05 B06	FAM FAM	Unkn Unkn		N/A N/A	
с	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			B07	FAM	Unkn		N/A	_
D	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			B08	FAM	Unkn		N/A	
E	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			C01	FAM	Unkn		N/A	
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			C02	FAM FAM	Unkn		N/A	
G	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk			C03 C04	FAM	Unkn Unkn		N/A N/A	
	UNK	UNK	OUK	OUK	UNK						C05	FAM	Unkn		N/A	
н							Neg	Pos			C06	FAM	Unkn		N/A	-
Comple	ted			Sca	an Mode: A	ll Channels	Plate T	ype : BR White Analys	is Mode: No Baseline	Subtraction						

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**.

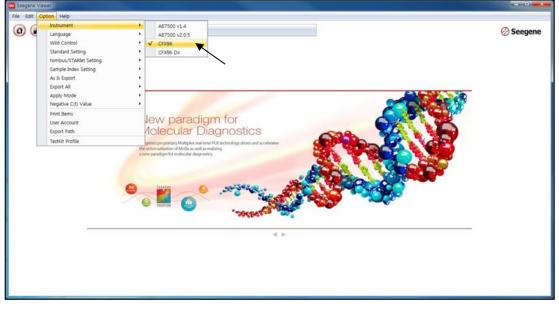


Fig. 13. Seegene Viewer

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

Ed																				
	6				PRODUCT							×						\bigotimes	Seeg	jen
Te	est - Quantita	ation Ct Result	ts,xlsx 🗙	±								\sim								
WE	ELL PLATE				•	WELL GR	APH						`							
1	123	4 5 6	7 8	9 10 1	1 12 🗸	FAM	HEX 🔽	Cal Red 6	10 🔽 Quas	ar 670										
C				000		0.9		-				0.9								
6		ÕÕÕ	ÕÕ	000		0.9						0.9								
ē		ŏŏŏ	ŏŏ	000		0.7						0.7								
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ā	i i i	ăăă	ŏĕ	002												-	-	-		-
2			žž			0.4						0.4								
2						0.3						0.2								
	566																			
						0.2			4.05	0.6 0.7		0.2				0.5	0.6	0.7		0.0
C	Jegative	Positive 4	Invalid	Combine		0.2	.1 0.2		.4 0.5 Cycle (Graph		0.8 0.9	0.2	0 0.1	0.2 0.	3 0.4 Cycl			0.7	0.8	0.9
) N			Invalid	Combine		0.2	.1 0.2		.4 0.5 Cycle (Graph		0.8 0.9	0.2	0 0.1	0.2 0.		0.5 e (Grap		0.7	0.8	0.9
) N	Negative		Invalid	Combine		0.2	.1 0.2				0.8 0.9	0.2	0 0.1		Cycl	e (Grap	h 2)			
			Invalid	Combine		0.2	.1 0.2				0.8 0.9	0.2	0 0.1			e (Grap	h 2)		0.8	
N API	PLY RESULT		Invalid	Combine	Туре	0.2			Cycle (Graph		0.8 0.9 Quasar 6	0.2	0 0.1	Pos	Cycl	e (Grap	h 2)			
N API	PLY RESULT	-				0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			rizon
	PLY RESULT	-	Well A01 B01		Type SAMPLE SAMPLE	0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			
	PLY RESULT	-	Well A01 B01 C01		Type SAMPLE SAMPLE SAMPLE	0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			rizon
	PLY RESULT	-	Well A01 B01 C01 D01		Type SAMPLE SAMPLE SAMPLE SAMPLE	0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			rizon
N N	PLY RESULT	-	Well A01 B01 C01 D01 E01		Type SAMPLE SAMPLE SAMPLE SAMPLE SAMPLE	0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			rizon
N API	PLY RESULT	-	Well A01 B01 C01 D01 E01 F01		Type SAMPLE SAMPLE SAMPLE SAMPLE SAMPLE SAMPLE	0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			rizon
N N API	PLY RESULT	-	Well A01 B01 C01 D01 E01		Type SAMPLE SAMPLE SAMPLE SAMPLE SAMPLE	0.2		(Cycle (Graph	1)		0.2		Pos	Cycl sitive Find	e (Grap	h 2)			rizon

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.

	ion Help																					
		0	- 1	Allpl	ex™ STI E	Essentia	l Assay	(8 strip)	l.												🔗 S	Seegen
	antitation Ct Re	sults, xis:	(× 🔳		- (. R)																	
WELL PLA						WELL																
1 2	3 4 5	67	8 9	10 11 1	2	FAM	🔽 HEX	🔽 Ca	al Red 6	610 🔽] Quasa	r 670										
					BFU BFU	500 500 400 300								UU	/i	1000 750						С
APPLY RE	Positive SULT	l Inva	id Con	mbine				10			0 (Graph		30		40	250	A01		10	Cycle	20 30 e (Graph 2)	
G O O O O O O O O O O O O O O O O O O O	-		id Co	mbine				· · · 10		Cycle	(Graph		30		40		0	_	Positive	Cycle Find	(Graph 2)	· · · ·
APPLY RE			lid Con	Type		100		C(t)			(Graph		30 UP	Cal Re C(t)	40		A01 0 Quasa	_		Cycle Find	(Graph 2)	 Horizor
APPLY RE Well Info	SULT	1) Inva	Name		UU	100 A0	M		мн	Cycle I	(Graph	1)		Cal Re	40 d 610	0	Quasa	r 670	Positive Quasa IC	Cycle Find (e (Graph 2)	 Horizor
Negative APPLY RE Well Info Sample I	SULT	1) Inva	Name	Туре	UU +	100 0 40 0 FAI	M	C(t)	MH -	Cycle HE C(t)	(Graph X MG	1) C(t)	UP	Cal Re C(t)	40 d 610 CT	0 - C(t)	Quasa	r 670 C(t)	Positive Quasa IC +	Cycle Find r 670 C(t)	e (Graph 2)	 Horizor
Negative Negative Well Info Sample I	SULT	Unva	Name	Type	UU +	FAI	M NG -	C(t) N/A	MH - -	HE C(t)	(Graph X MG	1) C(t) N/A	UP -	Cal Re C(t) N/A	40 d 610 CT +	0 - C(t) 34,23	Quasa TV -	r 670 C(t) N/A	Positive Quasa IC +	Cycle Find 670 C(t) 24,35	e (Graph 2) © Vertical (Auto Interpreta UU,CT	 Horizor
APPLY RE	SULT	Inva Well A01 B01	Name	Type SAMPLE SAMPLE	UU + + +	FAI 0 40 0 5 0 5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	M NG -	C(t) N/A N/A	MH 	HE C(t) N/A N/A	(Graph X MG -	1) C(t) N/A N/A	UP - -	Cal Re C(t) N/A N/A	40 d 610 CT + +	0 . C(t) 34,23 32,93	Quasa TV -	r 670 C(t) N/A N/A	Positive Quasa IC + +	Cycle Find 670 24.35 23.22	(Graph 2)	 Horizor
APPLY RE Well Info	SULT	Inva Well A01 B01 C01	Name	Type SAMPLE SAMPLE SAMPLE	UU + + +	FAI C(t) 33.03 34.80 33.28	M NG - -	C(t) N/A N/A	MH 	HE C(t) N/A N/A N/A	(Graph X MG -	1) C(t) N/A N/A	UP - -	Cal Re C(t) N/A N/A N/A	40 d 610 CT + +	0 C(t) 34,23 32,93 35,72	Quasa TV - -	670 C(t) N/A N/A	Positive Quasa IC + +	Cycle Find 670 C(t) 23,22 24,05	(Graph 2)	 Horizor
G Negative Negative Well Info	SULT	(1) Inva Well A01 B01 C01 D01	Name	Type SAMPLE SAMPLE SAMPLE SAMPLE	UU • • •	FAI 0 33.03 34.80 33.28 35.47	M NG - -	C(t) N/A N/A N/A	MH 	HE C(t) N/A N/A N/A	(Graph) X 	1) C(t) N/A N/A N/A	UP 	Cal Re C(t) N/A N/A N/A N/A	40 d 610 CT + + + +	C(t) 34,23 32,93 35,72 33,07	Quasa TV - - -	C(t) N/A N/A N/A N/A	Positive Quasa IC + + + +	Cycle Find 670 24.35 23.22 24.05 23.26	(Graph 2)	 Horizor

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:

					Seegene	Viewer F	Result		
Control	FAM	I (C _t)	HEX	(C _t)	Cal Red	610 (C _t)	Quasar	670 (C _t)	Auto Interpretation
	UU	NG	MH	MG	UP	СТ	ΤV	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 \rightarrow New \rightarrow Protocol to open Protocol Editor.

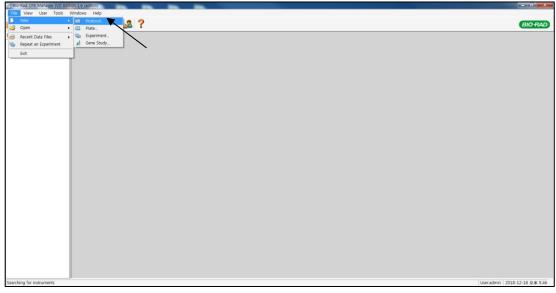


Fig. 1. Protocol Setup. Create a new protocol or load an existing protocol for the run



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

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

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

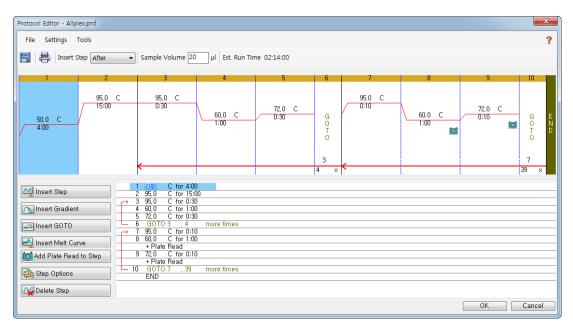


Fig. 2. Protocol Editor

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



- Run Setup × 📶 Protocol 💷 Plate 🕪 Start Run Express Load Create New... Select Existing... Selected Protocol • Allplex,prcl Edit Selected., Preview Est, Run Time: 02:14:00 (96 Wells-All Channels) Sample Volume: 20ul 95,0 C 15:00 95,0 C 0:30 95,0 C 0:10 72,0 C 0:10 72,0 C 0:30 60,0 C 1:00 G 0 T 0 60,0 C 1:00 G 0 T 0 ō 50,0 C 4:00 Ó Next >>
- 4) Click OK and save the protocol to open the Run Setup window.

Fig. 3. Run Setup: Protocol

B. Plate Setup

Run Set		Plate > :	Start Run									X
S	Create New, elect Existing ted Plate	~	`						Express Los QuickPlate_		Channels, pitd	•
	Plate_96 well:	s_All Channel	s,pltd								Edit Se	elected
Fluore	ophores:	FAM, H	IEX, Texas Re	ed, Cy5, Quas	sar 705			Plate Typ	e: BR Clear		Scan Mode	: All Channels
	1	2	3	4	5	6	7	8	9	10	11	12
A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
в	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
с	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
D	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
E	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
Gi	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
н	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk
										<< Pr	ev	Next >>

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.

	2 3	4 5 Select Fluorophores	6 7	8 9 10	11 12	🤖 Select Flu	orophores
3		Channel	Fluorophore	Selected Colo		Sample Type	
;			FAM			Load	Target Name <pre></pre>
)		2	HEX TET			Load	Sample Name
			Cal Gold 540 VIC				<none></none>
		3	ROX			Load	Replicate #
ì			Texas Red Cal Red 610				i' ate Series
1		4	Cy5 Quasar 670			000	nt Settings
		5	Quasar 705		_		Replicate #
				OK	Cancel	Clea	ar Wells

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).

Plate E	ditor - Test.p	itd	-								press land				x
File	Settings	Tools													
	Plate	Size	 Image: Image: Ima	Scan Mode 🖟	All Channels	-	al well a	iroups 🔤	2				<u>(1</u>	Plate Loading G	Guide
	Plate		· ·											_	
	-	ber Conventie		BR Clear	Unk	6 Unk	7 Unk	8 Unk	9	10	11	12	Select Flu	orophores	
A	HEX Cal Red 610 Quasar 670	HEX Cal Red 610 Quasar 670	HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670	FAM HEX Cal Red 610 Quasar 670					Sample Type	Unknown	-
в	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					Load FAM HEX	Target Name <none> <none></none></none>	•
с	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					✓ Cal Red 610 ✓ Quasar 670	<none></none>	•
D	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					Load	Sample Name <none> Replicate #</none>	•
Е	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unik FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670					Colle	1 ate Series ent Settings	÷
F	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670						Replicate # ar Wells	
G	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670	Unk FAM HEX Cal Red 610 Quasar 670							
н							Neg FAM HEX Cal Red 610 Quasar 670	Pos FAM HEX Cal Red 610 Quasar 670							
Pla	ate Type : Bl	R White											ок ОК	Cancel	

Fig. 6. Plate Setup

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

📶 Pr	rotocol	Plate 🕪										
	Create New,							G	Express Load			
	elect Existin	g										
	ted Plate											
'est, p 'revie											Edit Sel	ected
	phores:	FAM, H	IEX, Cal Red	610, Quasar	670			Plate Tvp	e: BR White		Scan Mode:	All Channel
	1	2	3	4	5	6	7	8	9	10	11	12
A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
в	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
С	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
D	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
G	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk				
н							Neg	Pos				

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.

Run Setup					
	💷 Plate 🕩 Start Run				
-Run Informati					
	Allplex.prcl Test.pltd				
Notes:	Test.pitu				*
Scop Model	All Channels				T
	Selected Block(s)				
	Block Name 4	Туре	Run Status	Sample Volume	ID/Bar Code
BR100		"96FX"	Idle	20	
E Select All	Blocks				
		pen Lid	Close Lid		
		pen Lid	Close Lid		
		ipen Lid	Close Lid		▶ Start Run
		pen Lid 📃 🔼	Close Lid		Start Run

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

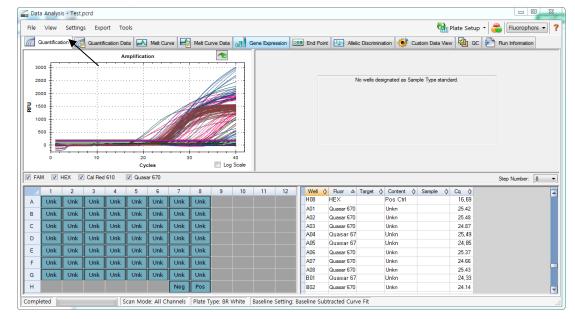
A. Create folders for data export

1) To save data for all of amplification curve detection 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

- 🕻 Data Analysis Test.pcrd 🐏 Plate Setup 🔹 츕 🛛 Fluorophore 👻 File View Export Tools Quantific Cq Determination Mo 🔄 Melt Curve Data 📶 Gene Expression 🔤 End Point 🕼 Alleic Discrimination 😻 Custom Data View 🖓 QC 🎥 Run Information ₽ Baseline Subtracted Analysis Mode Ø Baseline Subtracted Curve Fit Cycles to Analyze 1 8000 No wells designated as Sample Type standard Apply Fluorescence Drift Correction Raseline Threshold 7000 Trace Styles F 6000 **6** Plate Setup 5000 Include All Excluded Wells Mouse Highlighting 4000 Restore Default Window Layout 40 Log Scale Cycles FAM HEX Cal Red 610 Quasar 670 Step Number: 8 9 10 11 12
 Well
 Oral Plan
 Target
 Content
 Sample
 Cq
 O

 G08
 FAM
 Unkn
 N/A
 Unk Unk Unk Α Unk Unk Unk Unk Unk Neg Ctrl Pos Ctrl Unkn N/A N/A N/A H07 FAM в Unk Unk Unk Unk Unk Unk Unk Unk FAM H08 A01 с Unk Unk Unk Unk Unk Unk Unk Unk A02 A03 A04 HEX Unkn N/A Unk D Unk Unk Unk Unk Unk Unk Unk HEX N/A N/A Unkn Е Unk Unk Unk Unk Unk Unk Unk Unk Unkn A05 HEX Unkn N/A F Unk Unk Unk Unk Unk Unk Unk Unk A06 A07 HEX HEX Unkn Unkn N/A N/A G Unk Unk Unk Unk Unk Unk Unk Unk н Neg Pos A08 HEX Unkn N/A Scan Mode: All Channels | Plate Type: BR White | Baseline Setting: No Baseline Subtraction Completed
- 2) Select No Baseline Subtraction from Baseline Setting of Settings menu.

Fig. 10. No Baseline Subtraction



3) Select Seegene Export from Export menu.

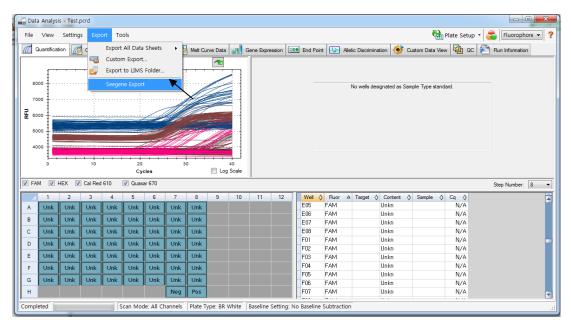


Fig. 11. Seegene Export

ile	View	Setting	is Expo	ort To	ols									e	Plate Setu	1p - qt	Fluoroph	ore 🔻
1 0	uantificat	ion 🜈	Quantif	ication Da	ta 🛃	Melt Cur	ve 🛃	Melt Cun	e Data	End Poir	nt 🔛 Al	lelic Discrimina	tion 🔅 C	ustom Data V	iew 🖄 Q	ic 🖹	Run Informatio	n
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80																		
												No wells desi	gnated as Sar	nple Type sta	ndard.			
70	00 †				••••••		A		Browse For Folder			— ×-						
60	n I								Choose destination folder									
60																		
50	∞ <u>†</u> .			-		and a	11		Desktop			Â						
40	. I.=						11		Libraries			=						
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	0		10		20		3	0	Network Sector Panel									
					Cy	cles			Recycle Bin									
FAI	4 🔽 H	HEX 🔽	Cal Red	610	V Quas	ar 670						-					Step Number:	8
	1	2	3	4	5	6	7	8	Make New Folder	ОК		Cancel	Content 🔇	Sample	♦ Cq ♦			
A	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Make New Folder	OK		Jancel	Jnkn		N/A			
3	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk		C01	FAM		Unkn		N/A			
-	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk		C02 C03	FAM FAM		Unkn Unkn		N/A N/A			
										C03	FAM		Unkn		N/A			
	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk		C05	FAM		Unkn		N/A			
:	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk		C06	FAM		Unkn		N/A			
F	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk		C07	FAM		Unkn		N/A			
	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk		C08	FAM		Unkn		N/A			
	OIIK	ONK	OTIK	OIK	ONK	ONK				D01	FAM		Unkn		N/A			
G H							Neg	Pos		D02	EAM		Unkn		N/A			

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.

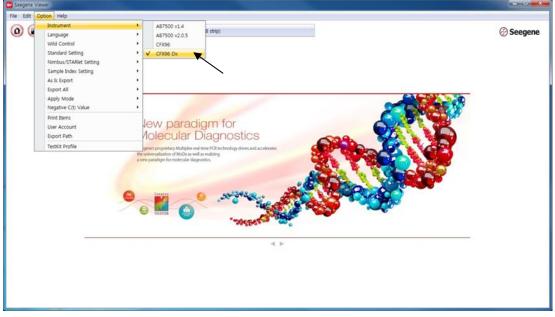


Fig. 13. Seegene Viewer

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

) 🕝 🖪				PRODUCT									6	🦻 Seeg	en
Test - Quantit	ation Ct Result	ts.xlsx 🗙	E												
WELL PLATE				•	WELL GRAPH				`						
1 2 3	4 5 6	7 8	9 10 11	I 12 💽	FAM 📝 HEX	🔽 Cal Red 61	0 👿 Quasar 670								
			000		0.9				0.9						
	000	00	000		0.8				0.9						
000		00	000		0.7				0.7						
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	ŏŏŏ	ŏŏ	000		0.3				0.3						
			000		0.2				0.2						
					0.1				0.1						
					0.1	0.2 0.3 0	4 0.5 0.6 0.7	0.8 0.9		0.1 0.2	0.3 0.4	0.5	0.6 0.7	0.8	0.9
	Positive () Invalid	Combine		0.1		4 0.5 0.6 0.7 ycle (Graph 1)	0.8 0.9	0.1	0.1 0.2	0.3 0.4 Cy	0.5 cle (Grapt		0.8). 9
Negative		Invalid	Combine		0.1			0.8 0.9	0.1	0.1 0.2				0.8).9
Negative		Invalid	Combine		0.1			0.8 0.9	0.1	0.1 0.2	Cy	cle (Graph	12)		
Negative	Т				0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)	0.8 cal () Hoi	
Negative APPLY RESUL Well Info Sample No		Well	Name	Туре	0.1			0.8 0.9	0.1		Cy	cle (Graph	12)		
Negative APPLY RESUL Well Info Sample No	Т	Well A01	Name	SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		izon
Negative	Т	Well A01 B01	Name	SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		
APPLY RESUL Well Info	Т	Well A01 B01 C01	Name	SAMPLE SAMPLE SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		izon
Negative APPLY RESUL Well Info Sample No	Т	Well A01 B01 C01 D01	Name	SAMPLE SAMPLE SAMPLE SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		izon
APPLY RESUL Well Info	Т	Well A01 B01 C01	Name	SAMPLE SAMPLE SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		izon
Negative	Т	Well A01 B01 C01 D01 E01	Name	SAMPLE SAMPLE SAMPLE SAMPLE SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		izon
Negative	Т	Well A01 B01 C01 D01 E01 F01	Name	SAMPLE SAMPLE SAMPLE SAMPLE SAMPLE	0.1	c	ycle (Graph 1)		0.1		Cy Positive Fin	cle (Graph	12)		izon

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.

Edit Option	п нер																					
) 🕝 (n		Allpl	ex™ STI E	Essentia	l Assay	(8 strip))												\bigotimes	Seeger
Test - Quar	ntitation Ct Res	ults, xls:	< × 🔳																			
WELL PLATE					•	WELL	GRAPH															
1 2 3	3 4 5	67	8 9	10 11 1	2	FAM	🔽 HEX	< 🔽 C	al Red 6	610 🔽	Quasa	r 670										
						+							-		-		1					
			Ö 🔘 (6	500									1	1000						1
			ă ă i	000	5	500								UU	4	750					/	ю
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					2	200								/	_							
						200								/		250			_			
														_ ui			A01					IQ1
						100	01								-		A01					
	Positius					100)1	1	0				30		40		A01		10		20 30	
	Positive) ()) Inva		mbine		100	0.1	1			0 (Graph		30	u	-				10		20 30 e (Graph 2)	
Negative (-) Inva		mbine		100	01	1					30		-				10			
Negative (-	l Inva		mbine		100	01	1					30	 	-						(Graph 2)	
Negative APPLY RESL Well Info	JLT	-	lid 🔳 co			100		1			Graph		30	Cal Re	40			_		Cycle e Find	(Graph 2)	
Negative APPLY RESL Well Info	JLT	U Inva		Type				C(t)		Cycle	Graph		30 UP		40		0	_	Positiv	Cycle e Find	(Graph 2)	
Negative (APPLY RESL Well Info Sample No	JLT	-	lid 🔳 co		UU	100 A0	M		_	Cycle I	(Graph	1)		Cal Re	40 d 610	0	Quasa	r 670	Positive Quasa	Cycle e Find (r 670	(Graph 2)	al Horizo etation
Negative (APPLY RESL Well Info Sample No	JLT	Well	lid 🔳 co	Туре	UU +	100 0 40 0 FAI	M	C(t)	мн	Cycle HE C(t)	(Graph X MG	1) C(t)	UP	Cal Re C(t)	40 d 610 CT	0 C(t)	Quasa	r 670 C(t)	Positive Quasa IC	Cycle e Find r 670 C(t)	(Graph 2)	al O Horizo etation
Negative Negative Well Info Sample No	JLT	Well A01	lid 🔳 co	Type	UU •	FAI	M NG -	C(t)	MH -	Cycle HE C(t) N/A	(Graph X MG	1) C(t) N/A	UP -	Cal Re C(t) N/A	40 d 610 CT +	0 C(t) 34,23	Quasa TV -	r 670 C(t) N/A	Positiw Quasa IC +	Cycle e Find r 670 C(t) 24,35	e (Graph 2)	al O Horizo
Negative APPLY RESL Well Info	JLT	Well A01 B01	lid 🔳 co	Type SAMPLE SAMPLE	UU + +	FAI 0 40 0 5 0 5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	M NG -	C(t) N/A N/A	MH - -	HE C(t) N/A N/A	(Graph) X MG -	1) C(t) N/A N/A	UP - -	Cal Re C(t) N/A N/A	40 d 610 CT + +	0 C(t) 34,23 32,93	Quasa TV -	r 670 C(t) N/A N/A	Positive Quasa IC +	Cycle e Find r 670 C(t) 24,35 23,22	e (Graph 2) Q Vertica Auto Interpre UU.CT UU.CT UU.CT	al O Horizo
Negative Neg	JLT	Well A01 B01 C01	lid 🔳 co	Type SAMPLE SAMPLE SAMPLE	UU • •	FAI C(t) 33.03 34.80 33.28	M NG - -	C(t) N/A N/A	MH - -	HE C(t) N/A N/A	(Graph) X MG -	1) C(t) N/A N/A	UP - -	Cal Re C(t) N/A N/A N/A	40 d 610 CT + +	0 C(t) 34,23 32,93 35,72	Quasa TV - -	670 C(t) N/A N/A	Positive Quasa IC + +	Cycle Find r 670 C(t) 23,22 23,22 24,05	e (Graph 2) Caraph 2 Car	al O Horizo
Negative APPLY RESL Well Info	JLT	Well A01 B01 C01 D01	lid 🔳 co	Type SAMPLE SAMPLE SAMPLE SAMPLE	UU .	FAI 0 33.03 34.80 33.28 35.47	M NG - - -	C(t) N/A N/A N/A	MH - - -	HE C(t) N/A N/A N/A	(Graph) X MG - - -	1) C(t) N/A N/A N/A	UP 	Cal Re C(t) N/A N/A N/A N/A	40 d 610 CT + + + +	0 C(t) 34,23 32,93 35,72 33,07	Quasa TV - - -	C(t) N/A N/A N/A N/A	Positive Quasa IC + + +	Cycle Find r 670 24,35 23,22 24,05 23,26	e (Graph 2)	al Horizo etation

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:

		Seegene Viewer Result									
Control	FAM (C _t)		HEX	HEX (Ct)		Cal Red 610 (C _t)		670 (C _t)	Auto Interpretation		
	UU	NG	MH	MG	UP	СТ	ΤV	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	Ana	lyte		
Fluorophore	Graph 1	Graph 2		
FAM	Ureaplasma urealyticum	Neisseria gonorrhoeae		
17.001	(UU)	(NG)		
HEX	Mycoplasma hominis	Mycoplasma genitalium		
TIEX .	(MH)	(MG)		
Cal Red 610	Ureaplasma parvum	Chlamydia trachomatis		
Carried 010	(UP)	(CT)		
Quasar 670	Trichomonas vaginalis	Internal Control		
Quasal 070	(TV)	(IC)		

2. Interpretation of Results

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



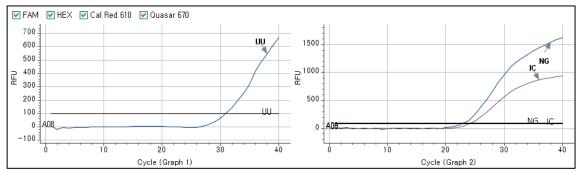
Target	Target Result		Interpretation					
Graph 1	Graph 2	IC Result	Interpretation					
+	-		Target Nucleic acid, Detected					
-	+	+						
+	+							
+	-		Target Nucleic acid, Detected*					
-	+	-	- Additional STI targets that were not detected may be					
+	+		present.					
-	-	+	Target Nucleic acid, 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 TROUBLESHOOTING.

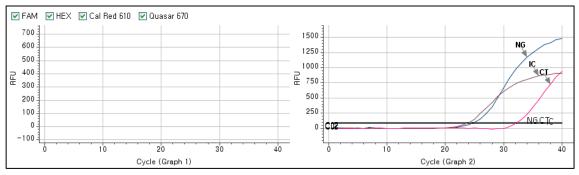


3. Application to Clinical Samples

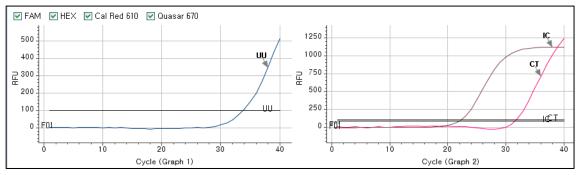
Sample 1



Sample 2



Sample 3



Comple		FA	M			HE	EX			Cal Re	ed 610		Quas	ar 670	Quas	ar 670	Auto
Sample	UU	C(t)	NG	C(t)	мн	C(t)	MG	C(t)	UP	C(t)	СТ	C(t)	τv	C(t)	IC	C(t)	Interpretation
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 [™] S	TI Essential Assay
OBSERVATION	PROBABLE CAUSES	SOLUTION
	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.
No signal	Incorrect storage or past expiration date of the test kit	Please check the storage conditions (See page 10) and the expiration date (refer to label) of the test kit and use a new kit if necessary.
	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.
	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.
No Internal Control signal	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 [™] ST	T 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.
	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.
Dutative false	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid.
Putative false negative or no signal observed in Positive	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.
Control	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 was 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	КСТС	12409	Not Detected
29	Acinetobacter ursingii	КСТС	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	КСТС	15240	Not Detected
34	Bacteroides caccae	ATCC	43185	Not Detected
35	Bacteroides fragilis	КСТС	5013	Not Detected
36	Bacteroides ovatus	КСТС	5827	Not Detected
37	Bacteroides vulgatus	ATCC	8482	Not Detected
38	Bacteroides xylanisolvens	КСТС	15192	Not Detected
39	Bifidobacterium adolescentis	КСТС	3216	Not Detected
40	Bifidobacterium longum	КСТС	3421	Not Detected
41	Bifidobacterium minimum	КСТС	3273	Not Detected
42	Campylobacter rectus	КСТС	5636	Not Detected
43	Candida albicans	ATCC	10231D-5	Not Detected
44	Candida dubliniensis	КСТС	17427	Not Detected
45	Candida glabrata	KCCM	50044	Not Detected
46	Candida krusei	KCCM	11426	Not Detected
47	Candida lusitaniae	КССМ	50541	Not Detected
48	Candida metapsilosis	ATCC	96144D	Not Detected
49	Candida orthopsilosis	ATCC	96139	Not Detected
50	Candida parapsilosis	КСТС	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	КСТС	3269	Not Detected
56	Corynebacterium diphtheriae	КСТС	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	КСОМ	1657	Not Detected
63	Gardnerella vaginalis	КСТС	5097	Not Detected
64	Haemophilus ducreyi	ATCC	700724D-5	Not Detected
65	Haemophilus influenzae	КССМ	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	КСТС	3140	Not Detected
77	Lactobacillus amylovorus	KCTC	3179	Not Detected
78	Lactobacillus brevis	КСТС	3498	Not Detected
79	Lactobacillus casei	KCTC	3260	Not Detected
80	Lactobacillus crispatus	КСТС	5054	Not Detected
81	Lactobacillus delbrueckii subsp. Delbrueckii	КСТС	13730	Not Detected
82	Lactobacillus fermentum	КСТС	3112	Not Detected
83	Lactobacillus gallinarum	КСТС	5048	Not Detected
84	Lactobacillus gasseri	КСТС	3163	Not Detected
85	Lactobacillus helveticus	КСТС	15060	Not Detected
86	Lactobacillus iners	CCARM	123	Not Detected
87	Lactobacillus intestinalis	КСТС	5052	Not Detected
88	Lactobacillus jensenii	КСТС	5194	Not Detected
89	Lactobacillus johnsonii	КСТС	3801	Not Detected
90	Lactobacillus kefiranofaciens	КСТС	5075	Not Detected
91	Lactobacillus oris	КССМ	40993	Not Detected
92	Lactobacillus parabuchneri	КСТС	3503	Not Detected



93 Lactobacillus pentosus KCTC 3120 Not Detected 94 Lactobacillus pentosus ATCC 700934 Not Detected 95 Lactobacillus reuteri KCTC 3679 Not Detected 96 Lactobacillus stammosus KCCM 32405 Not Detected 97 Lactobacillus stammosus KCTC 3600 Not Detected 98 Lactobacillus stammosus KCTC 3600 Not Detected 99 Lactobacillus subsp. Salicinius KCTC 3657 Not Detected 100 Lactobacillus vaginalis KCTC 3515 Not Detected 101 Mobiluncus curitsii ATCC 35241 Not Detected 102 Mobiluncus mulieris ATCC 35243 Not Detected 103 Moraxella catarnhalis KCCM 42706 Not Detected 104 Mycoplasma felis Cole et al. ATCC 23838 Not Detected 105 Mycoplasma penicaptivi Hill ATCC 33552 Not Detected 106 Mycoplasma pumonia ATCC 19612 Not Detected					
95Lactobacillus reuteriKCTC3679Not Detected96Lactobacillus rhamnosusKCCM32405Not Detected97Lactobacillus salivarius subsp. SaliciniusKCTC3600Not Detected98Lactobacillus sanfrancisensisKACC12431Not Detected99Lactobacillus ultunensisKCTC5857Not Detected100Lactobacillus vaginalisKCTC3515Not Detected101Mobiluncus curitsiiATCC35241Not Detected102Mobiluncus mulierisATCC35241Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma delis Cole et al.ATCC23391Not Detected106Mycoplasma envisional envisionATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC19612Not Detected108Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Nelsseria leongataZMC801510Not Detected112Nelsseria flavescensCCARM9264Not Detected113Nelsseria flavescensATCC23970Not Detected114Nelsseria flavescensATCC19696Not Detected115Neisseria flavescensATCC19696Not Detected116Neisse	93	Lactobacillus pentosus	КСТС	3120	Not Detected
96Lactobacillus rhamnosusKCCM32405Not Detected97Lactobacillus salivarius subsp. SaliciniusKCTC3600Not Detected98Lactobacillus sanfrancisensisKACC12431Not Detected99Lactobacillus ultunensisKCTC5857Not Detected100Lactobacillus vaginalisKCTC3515Not Detected101Mobiluncus curitsiiATCC35241Not Detected102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma argininiATCC23391Not Detected106Mycoplasma felis Cole et al.ATCC33552Not Detected107Mycoplasma iowae Jordan et al.ATCC33552Not Detected108Mycoplasma pneumoniaATCC19612Not Detected109Mycoplasma pneumoniaATCC19526Not Detected111Neisseria flavescensATCC1110Not Detected112Neisseria flavescensATCC23970Not Detected113Neisseria flavescensATCC23970Not Detected114Neisseria flavescensATCC23970Not Detected115Neisseria flavescansATCC23970Not Detected116Neisseria flavescansATCC19696Not Detected117Neisseria	94	Lactobacillus plantarum	ATCC	700934	Not Detected
97Lactobacillus salivarius subsp. SaliciniusKCTC3600Not Detected98Lactobacillus vaginalisKACC12431Not Detected99Lactobacillus vaginalisKCTC3515Not Detected100Lactobacillus vaginalisKCTC3515Not Detected101Mobiluncus curtisiiATCC35241Not Detected102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma argininiATCC23391Not Detected106Mycoplasma argininiATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC33552Not Detected108Mycoplasma pneumoniaATCC19531Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC19526Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC23970Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria flavescensATCC13096Not Detected116Neisseria mingitidisATCC13096Not Detected117Neisseria mingitidisATCC </td <td>95</td> <td>Lactobacillus reuteri</td> <td>КСТС</td> <td>3679</td> <td>Not Detected</td>	95	Lactobacillus reuteri	КСТС	3679	Not Detected
98Lactobacillus sanfrancisensisKACC12431Not Detected99Lactobacillus ultunensisKCTC5857Not Detected100Lactobacillus vaginalisKCTC3515Not Detected101Mobiluncus curtisiiATCC35241Not Detected102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma felis Cole et al.ATCC33552Not Detected106Mycoplasma neuroniaATCC15531Not Detected107Mycoplasma peumoniaATCC19526Not Detected108Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma pulmonisATCC19526Not Detected111Neisseria cinereaATCC19526Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC13120Not Detected114Neisseria lactamicaATCC23970Not Detected115Neisseria meningitidisATCC10632DNot Detected116Neisseria meningitidisATCC10752Not Detected117Neisseria mingitidisATCC11703Not Detected118Neisseria mingitidisATCC </td <td>96</td> <td>Lactobacillus rhamnosus</td> <td>KCCM</td> <td>32405</td> <td>Not Detected</td>	96	Lactobacillus rhamnosus	KCCM	32405	Not Detected
99Lactobacillus ultunensisKCTC5857Not Detected100Lactobacillus vaginalisKCTC3515Not Detected101Mobiluncus curtisiiATCC35241Not Detected102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma regininiATCC23391Not Detected106Mycoplasma felis Cole et al.ATCC33552Not Detected107Mycoplasma felis Cole et al.ATCC33552Not Detected108Mycoplasma neumoniaATCC15531Not Detected109Mycoplasma pneumoniaATCC19612Not Detected109Mycoplasma pulmonisATCC19526Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC13120Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC13120Not Detected114Neisseria lactamicaATCC23970Not Detected115Neisseria meningitidisATCC10632DNot Detected116Neisseria meningitidisATCC11703Not Detected117Neisseria mingitidisATCC14799D-5Not Detected118Neisseria meningitidisATCC<	97	Lactobacillus salivarius subsp. Salicinius	КСТС	3600	Not Detected
100Lactobacillus vaginalisKCTC3515Not Detected101Mobiluncus curtisiiATCC35241Not Detected102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma felis Cole et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma pneumoniaATCC19612Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC13120Not Detected114Neisseria lactamicaATCC23970Not Detected115Neisseria neningitidisATCC10696Not Detected116Neisseria meningitidisATCC19696Not Detected117Neisseria mucosaATCC19696Not Detected118Neisseria mucosaATCC14799D-5Not Detected119Neisseria polyaacchareaZMC804030Not Detected120Neisseria polyaacchareaZMC <td>98</td> <td>Lactobacillus sanfrancisensis</td> <td>KACC</td> <td>12431</td> <td>Not Detected</td>	98	Lactobacillus sanfrancisensis	KACC	12431	Not Detected
101Mobiluncus curtisiiATCC35241Not Detected102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma iowae Jordan et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma pneumoniaATCC19612Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC13120Not Detected114Neisseria lactamicaATCC23970Not Detected115Neisseria lactamicaATCC10696Not Detected116Neisseria meningitidisATCC19696Not Detected117Neisseria meningitidisKCCM41762Not Detected118Neisseria mucosaATCC19696Not Detected119Neisseria polyaacchareaZMC804030Not Detected120Neisseria siccaATCC <td>99</td> <td>Lactobacillus ultunensis</td> <td>КСТС</td> <td>5857</td> <td>Not Detected</td>	99	Lactobacillus ultunensis	КСТС	5857	Not Detected
102Mobiluncus mulierisATCC35243Not Detected103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma iowae Jordan et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma peumoniaATCC19531Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC13120Not Detected114Neisseria lactamicaATCC23970Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria meningitidisATCC700532DNot Detected117Neisseria meningitidisKCCM41562Not Detected118Neisseria mucosaATCC19696Not Detected119Neisseria mucosaKCCM11703Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria siccaATCC29256N	100	Lactobacillus vaginalis	КСТС	3515	Not Detected
103Moraxella catarrhalisKCCM42706Not Detected104Mycoplasma argininiATCC23838Not Detected105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma iowae Jordan et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma peumoniaATCC19531Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria flavescensCCARM9264Not Detected113Neisseria flavescensATCC13120Not Detected114Neisseria lactamicaATCC23970Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria meningitidisATCC700532DNot Detected117Neisseria meningitidisKCCM41562Not Detected118Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria polysacchareaZMC804030Not Detected122Neisseria siccaATCC19256Not Detected123Neisseria siccaATCC14799D-5Not Detected124Neisseria siccaZMC804030	101	Mobiluncus curtisii	ATCC	35241	Not Detected
104Mycoplasma argininiATCC23838Not Detected105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma iowae Jordan et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma pneumoniaATCC15531Not Detected109Mycoplasma pneumoniaATCC19612Not Detected1010Mycoplasma pulmonisATCC19526Not Detected111Neisseria cinereaATCC19526Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC20970Not Detected116Neisseria meningitidisATCC700532DNot Detected117Neisseria meningitidisATCC19696Not Detected118Neisseria mucosaATCC14690Not Detected119Neisseria mucosaKCCM11703Not Detected111Neisseria polysacchareaZMC804030Not Detected112Neisseria siccaATCC14799D-5Not Detected113Neisseria siccaZMC804030Not Detected114Neisseria siccaATCC19696Not Detected115Neisseria mucosaKCCM11703 <t< td=""><td>102</td><td>Mobiluncus mulieris</td><td>ATCC</td><td>35243</td><td>Not Detected</td></t<>	102	Mobiluncus mulieris	ATCC	35243	Not Detected
105Mycoplasma felis Cole et al.ATCC23391Not Detected106Mycoplasma iowae Jordan et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma pneumoniaATCC15531Not Detected109Mycoplasma pneumoniaATCC19612Not Detected101Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC19696Not Detected118Neisseria mucosaATCC19696Not Detected120Neisseria pelfavaATCC14799D-5Not Detected121Neisseria piccaZMC804030Not Detected122Neisseria siccaATCC29256Not Detected123Neisseria siccaZMC801754Not Detected124Neisseria siccaZMC49275Not Detected	103	Moraxella catarrhalis	KCCM	42706	Not Detected
106Mycoplasma iowae Jordan et al.ATCC33552Not Detected107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma pneumoniaATCC15531Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria meningitidisATCC700532DNot Detected117Neisseria mucosaATCC19696Not Detected118Neisseria mucosaATCC14799D-5Not Detected120Neisseria perflavaATCC14799D-5Not Detected121Neisseria polysacchareaZMC804030Not Detected122Neisseria siccaATCC14799D-5Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaATCC49275Not Detected	104	Mycoplasma arginini	ATCC	23838	Not Detected
107Mycoplasma leonicaptivi HillATCC49890Not Detected108Mycoplasma pneumoniaATCC15531Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria flavescensATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria mucosaATCC19696Not Detected120Neisseria perflavaATCC14799D-5Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	105	<i>Mycoplasma felis</i> Cole et al.	ATCC	23391	Not Detected
108Mycoplasma pneumoniaATCC15531Not Detected109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria flavescensATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected120Neisseria mucosaATCC19696Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	106	<i>Mycoplasma iowae</i> Jordan et al.	ATCC	33552	Not Detected
109Mycoplasma pulmonisATCC19612Not Detected110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria flavescensATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria lactamicaZMC801752Not Detected118Neisseria meningitidisATCC700532DNot Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaZMC801754Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	107	Mycoplasma leonicaptivi Hill	ATCC	49890	Not Detected
110Mycoplasma spumansATCC19526Not Detected111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria flavescensATCC13120Not Detected116Neisseria lactamicaATCC23970Not Detected117Neisseria lactamicaZMC801752Not Detected118Neisseria meningitidisATCC700532DNot Detected119Neisseria mucosaATCC19696Not Detected120Neisseria perflavaATCC14799D-5Not Detected121Neisseria polysacchareaZMC804030Not Detected122Neisseria siccaATCC29256Not Detected123Neisseria siccaZMC801754Not Detected124Neisseria siccaZMC49275Not Detected	108	Mycoplasma pneumonia	ATCC	15531	Not Detected
111Neisseria cinereaATCC14685Not Detected112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected120Neisseria mucosaATCC19696Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	109	Mycoplasma pulmonis	ATCC	19612	Not Detected
112Neisseria elongataZMC801510Not Detected113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	110	Mycoplasma spumans	ATCC	19526	Not Detected
113Neisseria flavescensCCARM9264Not Detected114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria perflavaATCC14799D-5Not Detected121Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	111	Neisseria cinerea	ATCC	14685	Not Detected
114Neisseria flavescensATCC13120Not Detected115Neisseria lactamicaATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	112	Neisseria elongata	ZMC	801510	Not Detected
115Neisseria lactamicaATCC23970Not Detected116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	113	Neisseria flavescens	CCARM	9264	Not Detected
116Neisseria lactamicaZMC801752Not Detected117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	114	Neisseria flavescens	ATCC	13120	Not Detected
117Neisseria meningitidisATCC700532DNot Detected118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	115	Neisseria lactamica	ATCC	23970	Not Detected
118Neisseria meningitidisKCCM41562Not Detected119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	116	Neisseria lactamica	ZMC	801752	Not Detected
119Neisseria mucosaATCC19696Not Detected120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	117	Neisseria meningitidis	ATCC	700532D	Not Detected
120Neisseria mucosaKCCM11703Not Detected121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	118	Neisseria meningitidis	KCCM	41562	Not Detected
121Neisseria perflavaATCC14799D-5Not Detected122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	119	Neisseria mucosa	ATCC	19696	Not Detected
122Neisseria polysacchareaZMC804030Not Detected123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	120	Neisseria mucosa	KCCM	11703	Not Detected
123Neisseria siccaATCC29256Not Detected124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	121	Neisseria perflava	ATCC	14799D-5	Not Detected
124Neisseria siccaZMC801754Not Detected125Neisseria subflavaATCC49275Not Detected	122	Neisseria polysaccharea	ZMC	804030	Not Detected
125 Neisseria subflava ATCC 49275 Not Detected	123	Neisseria sicca	ATCC	29256	Not Detected
	124	Neisseria sicca	ZMC	801754	Not Detected
126 Neisseria subflava ZMC 804298 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	КСТС	15021	Not Detected
129	Prevotella bivia	КСТС	5454	Not Detected
130	Prevotella buccalis	КСТС	5496	Not Detected
131	Prevotella disiens	КСТС	5499	Not Detected
132	Prevotella intermedia	КСТС	5692	Not Detected
133	Prevotella melaninogenica	КСТС	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	КСТС	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 (\geq 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.

	Standard o	Genomic DNA	
Organism	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 10º CFU/mI	10 ¹
Mycoplasma hominis	ZeptoMetrix 0804011	2.69 x 10 ³ CCU/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 10º 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 AllplexTM 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, \geq 100.00% for Low positive samples and \geq 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 analytes for repeatability study: 100.00% for Moderate positive samples, 100.00% for Low positive samples and $\geq 2.50\%$ for High negative samples. The results were satisfied with the criteria, thus confirming the repeatable performances of AllplexTM 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 proven in diagnosing seven STI analytes, as the results satisfy the success criteria.

	PPA (compared to reference		NPA (compared to reference			Agreement			
Analyte	(compared to reference assay)			(compared to reference assay)					
	TP/ (TP+FN)	%a)	95% CI ^{c)}	TN/ (TN+FP)	% b)	95% CI ^{c)}	(TP+TN)/ Total	%d)	95% CI ^{c)}
Ureaplasma	434/436	99.54	98.35 ~ 99.94	1578	99.62	99.18 ~ 99.86	2012	99.60	99.22 ~ 99.83
urealiticum (UU)		00.01		/1584			/2020		
Neisseria	188/189	99.47	97.09 ~ 99.99	1827	99.78	99.44 ~ 99.94	2015	99.75	99.42 ~ 99.92
gonorrhoeae (NG)	100/100	59 99.47	97.09 ~ 99.99	/1831	99.70	99.44 * 99.94	/2020	99.13	99.42 · 99.92
Mycoplasma	344/346	99.42	97.93 ~ 99.93	1667	99.58	99.14 ~ 99.83	2011	99.55	99.16 ~ 99.80
hominis (MH)	344/340	544/540 99.42	97.95 ~ 99.95	/1674	99.00 99.14 ~ 99.00	99.14 ~ 99.03	/2020	99.00	99. IO ~ 99.00
Mycoplasma	263/263	100.00	98.61 ~ 100.00	1749	99.54	99.11 ~ 99.80	2012	00.00	00.00.00.00
genitalium (MG)	203/203	100.00	96.61 ~ 100.00	/1757	99.04	99.11 ~ 99.80	/2020	99.60	99.22 ~ 99.83
Ureaplasma	540/500	00.00	00.70 00.00	1492	400.00	00.75 400.00	2011	00.55	00.40, 00.00
parvum (UP)	519/528	98.30	96.79 ~ 99.22	/1492	100.00	99.75 ~ 100.00	/2020	99.55	99.16 ~ 99.80
Chlamydia	001/000	00.00	07.00 00.00	1756	00.00	00.50 00.00	2017	00.05	00.57 00.07
trachomatis (CT)		99.62 97.89 ~ 99.99	/1758	99.89 9	99.59 ~ 99.99	/2020	99.85	99.57 ~ 99.97	
Trichomonas	100/100	00.44	00.75 00.00	1851	400.00	00.00 400.00	2019	00.05	00.70 400.00
vaginalis (TV)	vaginalis (TV)		99.41 96.75 ~ 99.99		100.00	99.80 ~ 100.00	/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)
Ĩ	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
<u> </u>	Caution
Σ	Contains sufficient for <n> tests</n>
UDI	Unique Device Identifier



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*
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	Allplex [™] Bacterial Vaginosis Assay	100 rxns
SD10159X	Allplex [™] Bacterial Vaginosis <i>plus</i> Assay	100 rxns
SD9400Y	Allplex™ CT/NG/MG/TV Assay	50 rxns
SD9400X	Allplex [™] CT/NG/MG/TV Assay	100 rxns*
SD10169Y	Allplex [™] MG & AziR Assay	50 rxns
SD10170X	Allplex [™] MG & AziR Assay	100 rxns*

* For use with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet only

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*
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**

* For use with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet only

** 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 produc	ts	
SG1701	Ribo_spin vRD (Viral RNA/DNA Extraction Kit)	50 preps
Automated extract	tion 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
EX00003P	STARMag 96 UniPlate	96T / 1box
EX00004T	STARMag 96 UniTube	96T / 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