

INSTRUCTIONS FOR USE

NeoPlex™ STI-7 PLUS Detection Kit

REF

GM3600

Table of contents

1. INTENDED USE.....	1
2. PRINCIPLE OF ASSAY.....	1
3. KIT CONTENTS.....	1
4. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS.....	1
5. KIT STORAGE AND STABILITY.....	1
6. WARNINGS AND PRECAUTIONS.....	1
7. TEST PROCEDURE.....	2
8. INTERPRETATION OF TEST RESULTS.....	3
9. QUALITY CONTROL.....	3
10. TROUBLE SHOOTING.....	3
11. PERFORMANCE CHARACTERISTICS.....	4
12. LIMITATION OF TEST.....	5
13. SYMBOLS.....	6
Appendix. PCR Instrument Operation.....	7

1. INTENDED USE

The 'NeoPlex™ STI-7 PLUS Detection Kit' is a qualitative *in vitro* test for the simultaneous detection and confirmation of seven(7) sexually-transmitted infection(STI) causing pathogens including *Candida albicans(CA)*, *Gardnerella vaginalis(GV)*, *Treponema pallidum (TP)*, *Haemophilus ducreyi(HD)*, *Herpes simplex virus-1(HSV-1)*, *Herpes simplex virus-2(HSV-2)* and *Streptococcus agalactiae(GBS)* from urine or vaginal swab specimens.

2. PRINCIPLE OF ASSAY

'NeoPlex™ STI-7 PLUS Detection Kit' is based on two major processes, isolation of DNA from specimens and multiplex real-time amplification. STI-causing pathogens DNA is extracted from the specimens, amplified in multiplex real-time PCR and detected using fluorescent reporter dye probes specific for STIs-causing pathogens DNA and Internal Control. Internal Control(IC) serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

3. KIT CONTENTS

The 'NeoPlex™ STI-7 PLUS Detection Kit' components are shown in the table below.

Contents	Volume(96T)	Storage condition	Shelf life
4X NeoPlex PCR Master Mix	500 µL x 1 Vial		
4X STI-7 PLUS PPM	500 µL x 1 Vial		12 months (Before opening)
STI-7 PLUS Positive Control(PC)	50 µL x 1 Vial	Upper limit - 20°C	
STI-7 PLUS Internal Control(IC)	1 mL x 1 Vial		6 months (After opening)
DW(DNase-free Water)	1 mL x 1 Vial		

4. COMPATIBLE INSTRUMENT

- CFX96™ Dx System (Bio-Rad, Cat No.1845097-IVD)

5. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS

- CFX96™ Dx System (BioRad, Inc., Cat No. 1845097-IVD) or equivalent
- 0.2 ml 8-Tube PCR Strips without Caps, low profile, white (BioRad, Inc., Cat No. TLS0851)
- Optical Flat 8-Cap Strips for PCR Tubes

(BioRad, Inc., Cat No. TCS0803)

- Multiplate™ 96-Well PCR Plates, low profile, unskirted, white (BioRad, Inc., Cat No. MLL9651)
- QIAamp DSP DNA Mini Kit (QIAGEN, Cat No.61304) or equivalent DNA extraction kit
- Pipettes set
- Micro Centrifuge
- Disposable powder-free gloves

6. KIT STORAGE AND STABILITY

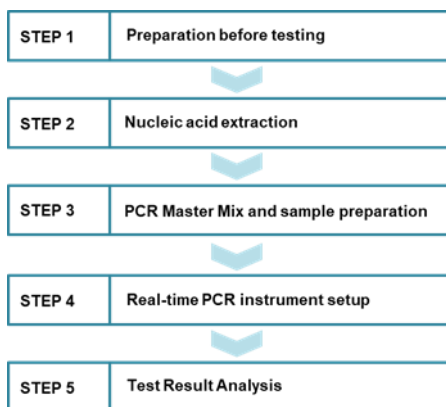
- Store the kit below -20°C(- 4°F).
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is one (1) year.
- Please use the reagents within six (6) months after opening.

7. WARNINGS AND PRECAUTIONS

1. This device is intended for *in vitro* use only. Do not use the device for other purposes.
2. Wear personal protective equipment, such as gloves and lab coats when handling NeoPlex™ STI-7 PLUS Detection Kit and/or specimens.
3. Do not smoke, drink or eat while handling NeoPlex™ STI-7 PLUS Detection Kit and/or samples.
4. Please be careful when handling samples to prevent infections of user and/or indirect contact to a person. Sample contains a risk of infections and unknown diseases.
5. Do not use reagents from different lots or from different tubes of the same lot.
6. If you do not frequently inspect the product, keep a kit in a refrigerator for a certain amount of time. Do not freeze/thaw over four times. Repeated frozen/thawed product may result in false negative and false positive results.
7. Be careful not to contaminate the product with nucleic acid extract, PCR product, positive control (PC, Positive Control). The use of filter tips is recommended to prevent contamination of the product.
8. It is recommended that the sample or the positive control (PC, Positive Control) contained in the product to be frozen and stored separately from the freezer storing the product.
9. Use the sterilized consumable laboratory supplies. Do not reuse it.
10. Add the extracted nucleic acid sample and positive control (PC, Positive Control) into the reaction solution in a space separate from the PCR reaction solution preparation space.
11. Before using, read this instruction for use carefully.

12. Use calibrated measuring tools. (e.g. pipette)
13. Please check the expiration date before using the reagent.
14. Keep Positive Control(PC, Positive Control) separately when using to avoid contamination
15. Before starting the PCR, make sure the lid is closed properly.
16. Dispose the product in accordance with local or national regulations.
17. Please consult with doctor about the test results.

8. TEST PROCEDURE



STEP 1. Preparation before testing

1) Preparation before testing

- A. Prepare all the devices and reagent before use.
- B. Place the kit under the room temperature at least 10 minutes before testing.



Do not freeze/thaw over four(4) times.

2) Specimen Collection, Transportation and Storage

- A. Specimens for use: Urine (first catch for the day recommended) and vaginal swab.
- B. It is recommended to process specimen within one(1) day after collection.
- C. Store specimens at 2-8 °C (35.6-46.4°F) for no longer than one (1) week. For pro-longed storage, Freeze at -20 ~ -80°C (-4°F ~ -112°F). The frozen specimen can be used for one(1) year.
- D. Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.



- Use only the specimen type listed in the instruction manual.
- The specimen volume should be above 1ml.
- Wear eye protection, laboratory coats and disposable gloves when handling specimens.
- Specimens should be stored under the storage conditions above. Otherwise, the wrong test results can be obtained.
- Sample information should be recorded to avoid confusion.

STEP 2. Nucleic acid extraction

After pre-treatment, DNA extraction can be done by automated purification system or using manual prep kits (QIAamp DSP DNA Mini Kit or equivalent).

1) Pre-treatment of the Specimen

Urine	Vaginal swab
Centrifuge 1mL of urine specimen for 10 minutes at 13,000 rpm.	Centrifuge 1mL of vaginal swab specimen with PBS for 10 minutes at 13,000 rpm.
Discard the supernatant. Re-suspend 1X PBS (1mL) on the vortexing.	Discard the supernatant. Re-suspend 1X PBS (1mL) on the vortexing.
Follow the manufacturer's protocol.	Follow the manufacturer's protocol.

2) Internal control (Optional)

The Internal Control (STI-7 PLUS Internal Control(IC)) is included in the kit. This allows the user to monitor the nucleic acid isolation procedure and the possibility of PCR inhibition.

Urine: Add 10 µL of STI-7 PLUS Internal Control(IC) to each sample solution mixture or directly to the lysis buffer.

3) DNA extraction

- 1) Add 10 µL of STI-7 PLUS Internal Control(IC) to a sample and add ATL buffer 180 µL provided by DNA extraction kit (QIAamp DSP DNA Mini Kit, by QIAGEN)
- 2) Incubate it at 56°C for 15 minutes.

* We recommend QIAamp DSP DNA Mini Kit or equivalent DNA extraction kit for nucleic acid extraction.

STEP 3. PCR Master Mix and sample preparation

1) Prepare the Master Mix

Contents	Volume per test
4X NeoPlex PCR Master Mix	5 µL
4X STI-7 PLUS PPM	5 µL
DW(DNase-free Water)	5 µL
Total Volume	15 µL

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

- 2) Vortex and briefly centrifuge the PCR Master Mix.
- 3) Place 15 µL aliquots of the PCR Master mix into 0.2 ml PCR tubes and close the lids.
- 4) Add 5 µL of each nucleic acid sample to its respective tube.

Contents	1 test (Volume)
PCR Mixture Mix	15 µL
Nucleic acid sample	5 µL
Total Reaction Volume	20 µL

Multiplex Real-time PCR Reagents for 7 Sexually Transmitted Pathogens Detection For professional *in vitro* diagnostic use only



- It is recommended that the PCR mixture to be prepared just before use.
- Aerosol-resistant filter tips and tight gloves should be used when preparing samples.
- Take great care to avoid cross contamination.
- Defrost the reagents completely.
- Centrifuge the reagent tubes briefly to remove the drops from the inside of the lids.

5) Make the control amplification reactions.

- Negative Control(NC): Add 5 µL of DW(DNase-free Water) instead of nucleic acid samples to the tube.
- Positive Control(PC): Add 5 µL of STI-7 PLUS Positive Control(PC) instead of nucleic acid samples to the tube.



- Use a new pipette tip with each different sample.
- Avoid cross-contamination of PCR Master Mix and samples with Positive Control.
- For CFX96™ Dx System, do not label on the cap of the reaction tubes as fluorescence is detected through the cap.
- Centrifuge the PCR tube thoroughly for 30 seconds.

STEP 4. Real-time PCR instrument setup

1) Setting the PCR protocol.

PCR protocol should be set according to the table as below.

Segment	Tm(°C)	Time	Cycles
1	50	4 min	1
2	95	15 min	1
3	95	30 sec	40
4	63	1 min	
5	73	10 min	
6	55	30 sec	
7*	Melting curve 55 °C ~ 85 °C (0.5°C/5s)*		

* Segment 7: Melting curve measurement

STEP 5. Test result analysis

Test results should be interpreted according to the '9. INTERPRETATION OF TEST RESULTS' presented as below.

9. INTERPRETATION OF TEST RESULTS

For the analysis of the test result after PCR amplification, take the melting peak result (For CFX96™ Dx System check the 'Melt Peak' tab) and interpret the according to the following interpretation table.

1. Interpretation criteria for result analysis

Target	Dye	Melt Tm	Cut-off(RFU*)
CA	FAM	67 ± 1°C	≥ 100
GV	FAM	77 ± 1°C	≥ 100
TP	Cal Red 610	68 ± 1°C	≥ 100

HD	Cal Red 610	77 ± 1°C	≥ 100
HSV-1	HEX	67.5 ± 1°C	≥ 100
HSV-2	HEX	76.5 ± 1°C	≥ 100
GBS	Quasar 670	78 ± 1°C	≥ 100
IC	Quasar 670	63.5 ± 1°C	≥ 100

* RFU(-d(RFU)/dT): Relative fluorescence units

2. Interpretation of result

Target	IC	Result	
+	+	Positive	The sexually transmitted pathogen is detected
+	-	Invalid	If the nucleic acid DNA concentration is high in the sample, IC signal may be attenuated. Dilute the template nucleic acid in distilled water and repeat the PCR with the diluted nucleic acid
-	+	Not detected	The sexually transmitted pathogen is not detected.
-	-	Invalid	The negative (-) result of IC is the result of inhibition of PCR reaction due to the presence of a PCR inhibitor contained in the sample, and the sample is not suitable for the test. It is recommended to remove the PCR inhibitor and perform the DNA extraction again.

3. Application examples of clinical samples

No	FAM		HEX		Cal Red 610		Quasar 670		Interpretation
	CA	GV	HSV-1	HSV-2	TP	HD	GBS	IC	
Sample 1	+	-	-	-	-	-	-	+	CA detected
Sample 2	-	-	+	-	+	-	-	+	HSV-1, TP detected
Sample 3	-	+	-	+	+	-	-	+	GV, HSV-2, TP detected
Sample 4	+	+	+	-	-	-	-	+	CA, GV, HSV-1 detected
Sample 5	+	-	-	-	-	-	-	+	CA detected
Sample 6	-	-	-	-	-	-	-	+	Not detected
Sample 7	-	-	-	-	-	-	-	-	Invalid
Positive Control	+	+	+	+	+	+	+	+	Positive (Valid)
Negative Control	-	-	-	-	-	-	-	-	Negative (Valid)

10. QUALITY CONTROL

NeoPlex™ STI-7 PLUS Detection Kit includes STI-7 PLUS Positive Control(PC) and DW(DNase-free Water) set. DW(DNase-free Water) is used as negative control. For all runs, valid test results must be obtained for both Positive and Negative control for NeoPlex™ STI-7 PLUS Detection Kit. Positive Control result must be Positive (Valid). Negative Control result must be Negative (Valid). If the positive and negative control results are consistently invalid, contact us for technical assistance.

11. TROUBLE SHOOTING

1. If all signals are not detected.

Potential causes	Solution
Error in specimen collection	If both target and IC signal were not observed, recollect the specimen

Multiplex Real-time PCR Reagents for 7 Sexually Transmitted Pathogens Detection For professional *in vitro* diagnostic use only

Nucleic acid extraction failure	Carefully read the instruction for use of nucleic acid extraction kit and extract the nucleic acid from specimen again.
Incorrect PCR setting	Repeat the detection procedure with a correct setting
Incorrect PCR cycle or machine temperature	Check the PCR conditions and repeat the PCR under the correct setting if necessary
The fluorescence for data analysis does not comply with the protocol	Select the correct fluorescence for each target listed in this Instruction guide for data analysis
Leaving reagents at room temperature for a long time or incorrect storage condition	Check the storage conditions and the expiration date of the reagents and use a new kit
Presence of inhibitor	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid (If specimen is still present, restart from nucleic acid extraction procedure)
High load of pathogen's nucleic acid	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid

2. If the Negative Control signal is detected/False positive.

Potential causes	Solution
Presence of cross contamination	Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Use filter tips during the extraction procedure. Change tips among tubes. Repeat the nucleic acid extraction with the new set of reagents

3. If the Positive control signals is Negative/False negative.

Potential causes	Solution
Error in specimen collection	Recollect the specimen
Incorrect storage of the specimen	Recollect the specimen and repeat the whole process. Make sure the product is stored in recommended conditions.
Error in nucleic acid extraction	Re-extract the nucleic acid
Incorrect PCR setting	Repeat the PCR with corrected setting
Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers for nucleic acid containing tubes and make sure to add nucleic acid into correct PCR tubes during detection process.
Incorrect PCR mixture	Check whether all components are added or not (If you use to pre-composed premix, should be reduce sensitivity) Each reagent should be used after homogenization and spin down reagent tube before putting the real-time PCR

12. PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

1.1 Limit of Detection (LoD)

This study was conducted to determine the sensitivity by testing Liquid based cytology specimen. The proportion of positive results obtained from each concentration was subjected to 95% hit rate by probit analysis, and LoD of each target were obtained by performing 20 times of the tests.

Target	Specimen type	LoD
CA	Urine	7.60x10 ¹ cfu/ml
	Vaginal Swab	7.25x10 ¹ cfu/ml
GV	Urine	3.21x10 ¹ cfu/ml
	Vaginal Swab	3.29x10 ¹ cfu/ml
TP	Urine	2.43x10 ⁰ copies/ul
	Vaginal Swab	1.36x10 ¹ copies/ul
HD	Urine	2.26x10 ⁰ copies/ul
	Vaginal Swab	2.26x10 ⁰ copies/ul
HSV-1	Urine	1.72x10 ⁰ copies/ul
	Vaginal Swab	1.65x10 ⁰ copies/ul
HSV-2	Urine	1.32x10 ¹ cfu/ml
	Vaginal Swab	1.27x10 ¹ cfu/ml
GBS	Urine	1.18x10 ¹ cfu/ml
	Vaginal Swab	1.15x10 ¹ cfu/ml

1.2 Cut-off value

For the cut-off establishment, ΔRFU value was set to be 100 for all of our target and specimens.

2. Analytical Specificity

2.1 Interference

Total thirteen (13) substances, endogenous and exogenous source, were studied to determine their interfering effect and no interference reactions was found with the concentration as below.

The test concentration was selected referring the competitor devices on the market.

No	Same Type	Interfering substance	Concentration	Remark
1	Urine	Urea	10mg/dL	Endogenous source
2		Glucose	10mg/dL	Endogenous source
3		pH (acid)	pH4	Endogenous source
4		pH (alkaline)	pH9	Endogenous source
5		Blood	5%	Endogenous source
6		Bovine Serum Albumin (BSA)	10mg/dL	Endogenous source
7		Phosphate-buffered saline (PBS)	1%	Sample treatment media
8	Vaginal swab	Phosphate-buffered saline (PBS)	1%	Sample treatment media
9		Blood	5%	Endogenous source
10		Human genomic DNA	1ng	Endogenous source
11		Canesten Cream	0.5%	Exogenous source
12		NOBASON Cream	0.5%	Exogenous source
13		GYNO-BETADINE	0.5%	Exogenous source

2.2 Cross reactivity

For analytical specificity, three(3) times of cross reactivity studies using seven(7) different pathogens similar with STI-pathogens pathogens were performed. Additionally, total twenty-nine(29) other pathogens were studied. As a result, PCR amplification and cross reactivity were not observed with all the pathogens as below.

No	Strain No	Pathogen	Remark
1	ATCC VR-348B	<i>Chlamydia trachomatis</i>	STI
2	ATCC 49226	<i>Neisseria gonorrhoeae</i>	
3	ATCC 33530	<i>Mycoplasma genitalium</i>	
4	ATCC 30001	<i>Trichomonas vaginalis</i>	
5	VTCC 14027	<i>Mycoplasma hominis</i>	
6	ATCC 25285D-5	<i>Bacteroides fragilis</i>	Other pathogens
7	ATCC 700802D-5	<i>Enterococcus faecalis</i>	
8	ATCC 12228D-5	<i>Staphylococcus epidermidis</i>	
9	HPKTCC B3204	<i>Neisseria meningitidis</i>	
10	HPKTCC B1834	<i>Neisseria sicca</i>	
11	ATCC VR-97	Influenza A virus (H1N1), A/FM/1/47	
12	ATCC VR-95	Influenza A virus (H1N1), A/PR/8/34	
13	ATCC VR-897	Influenza A virus (H1N1), A/New Jersey/8/76	
14	ATCC VR-546	Influenza A virus (H1N1), A/Denver/1/57	
15	ATCC VR-219	Influenza A virus (H1N1), A/NWS/33	
16	ATCC VR-823	Influenza B virus, B/Hong Kong/5/72	
17	ATCC VR-26	Human respiratory syncytial virus A, Long	
18	ATCC VR-955	Human respiratory syncytial virus B, 9320	
19	ATCC VR-3250SD	Quantitative Synthetic Human	

Multiplex Real-time PCR Reagents for 7 Sexually Transmitted Pathogens Detection For professional *in vitro* diagnostic use only

		metapneumovirus (hMPV) RNA
20	ATCC VR-94	Human parainfluenza virus 1 HPIV-1, C35
21	ATCC VR-1602	Human adenovirus 50
22	ATCC VR-284	Human Rhinovirus 14
23	ATCC VR-850	Human Coxsackievirus A 21
24	KBPV-VR-45	Parainfluenza virus 2
25	KBPV-VR-19	Echovirus 6
26	VIRCELL MBC090	Human coronavirus
27	ATCC 15531	Mycoplasma pneumoniae
28	VIRCELL MBC007	Bordetella parapertussis
29	VIRCELL MBC117	Moraxella catarrhalis
30	VIRCELL MBC031	Legionella pneumophila
31	ATCC 4357D-5	Lactobacillus acidophilus
32	ATCC 700669D-5	Streptococcus pneumoniae
33	ATCC 700928D-5	Escherichia coli
34	ATCC 13047D-5	Enterobacter cloacae
35	ATCC 700698D-5	Staphylococcus aureus
36	ATCC 12453D	Proteus mirabilis

2.3 Carry-over & Cross-contamination

This study was performed to evaluate the carry-over and potential cross contamination effect. High concentrated positive sample and negative control sample were cross tested using same PCR instrument, and 100% negative results (35/35) (95% CI: 90.11%-100%) for each negative specimen were determined, respectively.

3. Precision

3.1 Repeatability

To evaluate the repeatability of NeoPlex™ STI-7 PLUS Detection Kit, Repeatability test was performed by testing for twenty (20) different days, two(2) runs per day, two(2) cycles per run. Targets were set in three(3) levels of concentration, and 100% same results were obtained determining the repeatability. Samples were tested using high, medium, low concentrations of positive samples and negative control. We confirmed that every test results are met the acceptance criteria: within 10% of CV, 100% agreement and the repeatability of NeoPlex™ STI-7 PLUS Detection Kit is acceptable.

3.2 Reproducibility

To evaluate the reproducibility study, Reproducibility test was performed with four different conditions: for Between-lot (3 lots), Between-tester (3 testers), Between-instrument (3 instruments), and Between-site (3 sites).

We confirmed that every test results are met the acceptance criteria: within 10% of CV, 100% agreement and the reproducibility (Lot, Tester, Instrument, Site) of NeoPlex™ STI-7 PLUS Detection Kit is acceptable.

4. Clinical Evaluation

The clinical performance study was performed in the clinical laboratory, Samkwang Medical Laboratories, Seoul, Korea with the specimen collected from various sources, Such a hospitals, clinics, and health centers (medical check-up). The comparable CE-marked product already available on EU market was used as reference test.

For clinical sensitivity and specificity, the test results were analyzed with 2x2 table, and summarized as below:

4.1 Clinical Accuracy (Clinical Sensitivity & Specificity)

Target	Specimen type	Clinical sensitivity	Clinical specificity
CA	Vaginal swab	97.56% [95% CI 91.54-99.33]	100% [95% CI 99.04-100]
	Urine	100% [95% CI 91.62-100]	100% [95% CI 99.19-100]
	Total	98.38% [95% CI 94.31-99.56]	100% [95% CI 99.56-100]
GV	Vaginal swab	97.95% [95% CI 95.62-99.06]	100% [95% CI 97.97-100]
	Urine	98.51% [95% CI 94.76-99.59]	100% [95% CI 98.99-100]
	Total	98.13% [95% CI 96.36-99.05]	100% [95% CI 99.32-100]
HSV 1	Vaginal swab	97.50% [95% CI 87.12-99.56]	100% [95% CI 99.13-100]
	Urine	100% [95% CI 91.24-100]	100% [95% CI 99.19-100]
	Total	98.75% [95% CI 93.25-99.78]	100% [95% CI 99.58-100]
HSV 2	Vaginal swab	100% [95% CI 91.62-100]	100% [95% CI 99.13-100]
	Urine	97.56% [95% CI 87.4-99.56]	100% [95% CI 99.19-100]
	Total	98.79% [95% CI 93.49-99.79]	100% [95% CI 99.58-100]
TP	Vaginal swab	100% [95% CI 91.24-100]	100% [95% CI 99.13-100]
	Urine	97.5% [95% CI 87.12-99.56]	100% [95% CI 99.19-100]
	Total	98.75% [95% CI 93.25-99.78]	100% [95% CI 99.58-100]
GBS	Vaginal swab	98.38% [95% CI 91.41-99.71]	100% [95% CI 99.09-100]
	Urine	98.24% [95% CI 90.71-99.69]	100% [95% CI 99.16-100]
	Total	98.31% [95% CI 94.08-99.54]	100% [95% CI 99.56-100]
HD	Vaginal swab	97.5% [95% CI 87.12-99.56]	100% [95% CI 99.13-100]
	Urine	100% [95% CI 91.24-100]	100% [95% CI 99.19-100]
	Total	98.75% [95% CI 93.25-99.78]	100% [95% CI 99.58-100]

12. LIMITATION OF TEST

- Results from this test must be correlated with the clinical history, epidemiological data, and other data of the patient available to the clinician.
- If you do not use the samples and other specimens described in this manual, you may get inaccurate results.
- Although the results of this test are negative, it is not advisable to exclude the possibility that the infection is actually present.
- It is not excluded that this kit shows false positive results due to the presence of cross-contamination.
- False negative results may occur due to polymerase inhibition. STI-7 PLUS Internal Control(IC) may help to identify any substance existing in the specimens interfering with nucleic acid isolation and PCR amplification.
- This kit is for professional use only. Only trained healthcare provider can use this kit.

13. SYMBOLS

Catalogue number	Batch code	Date of manufacture	Use-by date	Distributor
<i>In vitro</i> diagnostic medical device	Upper limit of temperature	Caution	Consult instruction for use	Importer
Manufacturer	Contains sufficient for <n> tests	Authorized representative in the European Community	Conformity to European Directive 98/79/EC	Unique Device Identification

GeneMatrix Inc.
 Manufacturing site
 7F, #B, Korea Bio Park, 700, Daewangpangyo-ro,
 Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
 REPUBLIC OF KOREA
 Tel: +82-31-628-2045 Fax: +82-31-628-2002

MT Promedt Consulting GmbH
 Ernst-Heckel-Straße 7
 66386 St. Ingbert, Germany
 Tel: +49-6894-581020, Fax: +49-6894-581021

Issue date: 2022.07

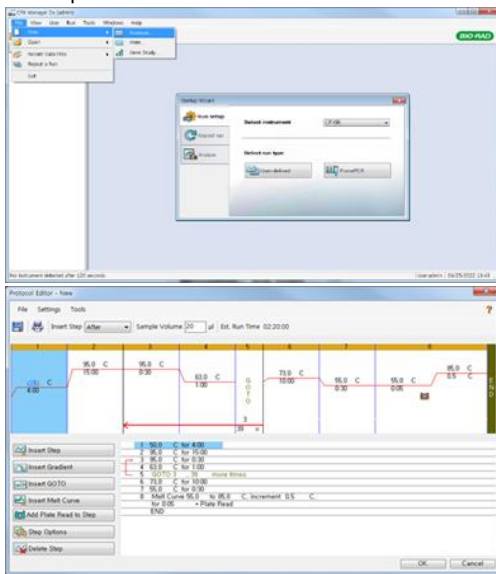
Multiplex Real-time PCR Reagents for 7 Sexually Transmitted Pathogens Detection
For professional *in vitro* diagnostic use only

Appendix. PCR Instrument Operation

1) CFX96™ Dx System (Bio-Rad)

1. Protocol Setup

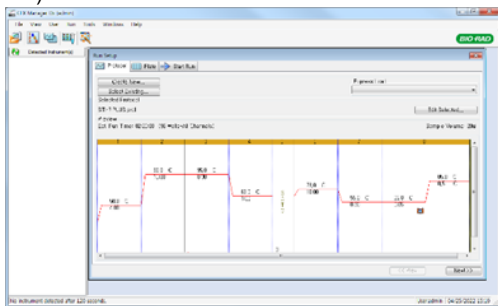
- Click File -> Protocol, Create a Protocol editor for PCR setup.
- The PCR condition is set as follows, and the sample volume set the 20 μ L.



Segment	Tm(°C)	Time	Cycles
1	50	4 min	1
2	95	15 min	1
3	95	30 sec	40
4	63	1 min	1
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 °C ~ 85 °C (5s /0.5°C)		

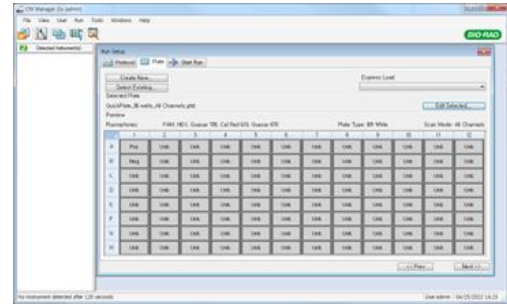
* Segment 7: Melting curve measurement

- After setting the PCR protocol, an Experiment Setup screen is created. Check the PCR protocol and click the "Next". (Or click the "Plate" tab)

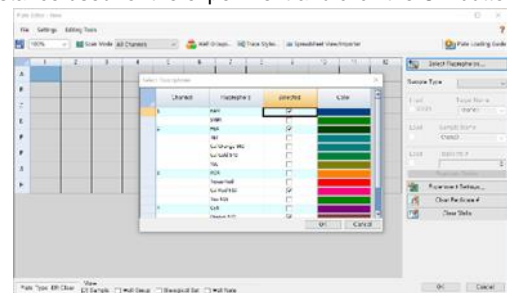


2. Plate Setup

- Click the "Create New" (or click the "Select Existing" load and existing plate for the experiment)



- Click the "Select Fluorophores". Select the check box (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) for the fluorescent substance used for the experiment and click the OK button.

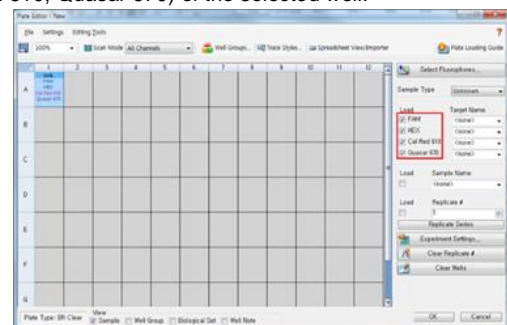


- Select wells and select Sample Type from the drop-down menu.

Sample Type

'Unknown' : Clinical samples
'Negative control'
'Positive control'

- Click the check box for the fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670) of the selected well.

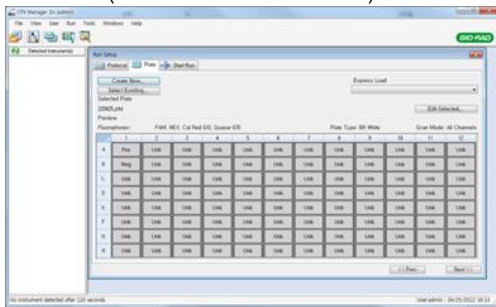


Multiplex Real-time PCR Reagents for 7 Sexually Transmitted Pathogens Detection For professional *in vitro* diagnostic use only

- 5 Click the "Settings" to set the plate type. (Settings -> Plate Type -> BR white)

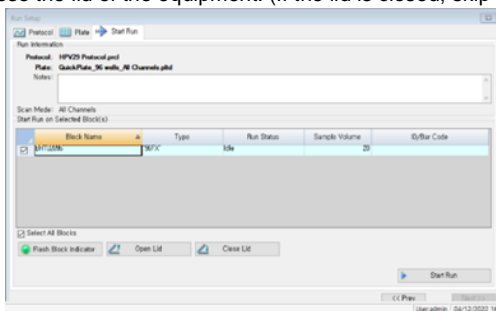


- 6 Click the "OK" and save a new Plate set-up file.
- 7 The Experimental Setup screen opens and checks the set plate. Click the "Next" (Or click the "Start Run" tab)



3. Start Run

- 1 In the Experiment Setup Start Run Tab screen, click the "Close Lid" to close the lid of the equipment. (If the lid is closed, skip the step)



- 2 Click the "Start Run".
- 3 The operating file is stored in the user's designated folder, and the equipment begins to operate.

4. Pre-setting for Data analysis

- 1 After the test, select the Melt curve to check the Melt Peak results.
- 2 Select each analytical fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) and set the threshold bar of Melt Peak to "0".

