

INSTRUCTION FOR USE

PCG® STIs FAST REAL-TIME PCR KIT

1. KIT CONTENT

REF	PR2200050	PR2200100
	50 tests	100 tests
STI Master Mix	1 x 1500 µL	3 x 1000 µL
STI-A	1 x 250 µL	1 x 500 µL
STI-B	1 x 250 µL	1 x 500 µL
STI-C	1 x 250 µL	1 x 500 µL
Positive Control	1 x 75 µL	1 x 150 µL
Negative Control	1 x 75 µL	1 x 150 µL

2. STORAGE AND TRANSPORTATION CONDITIONS

The kit is stored at -20°C (± 5°C), stable until the expiry date printed on the label and is recommended to be used within 8 weeks from the date of opening.

3. TECHNICAL SPECIFICATIONS

Regulatory Status	RUO
Intended use	Detection of nucleic acids from 12 types causative pathogen of sexually transmitted infection: <i>Neisseria gonorrhoeae</i> (NG), <i>Chlamydia trachomatis</i> (CT), <i>Trichomonas vaginalis</i> (TV), <i>Mycoplasma genitalium</i> (MG), <i>Gardnerella vaginalis</i> (GV), <i>Ureaplasma urealyticum</i> (UU), <i>Ureaplasma parvum</i> (UP), <i>Mycoplasma hominis</i> (MH), <i>Candida albicans</i> (CA), <i>Treponema pallidum</i> (TP), Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2)
Type of analysis	Qualitative
Validated specimen	Vaginal swab, Urine
Technology	Real-time PCR
Detection channels	FAM: NG, MG, GV HEX: CA, HSV-1, UP Texas Red: CT, TV, HSV-2 Cy5: MH, UU, TP Quasar 705: IC (Internal Control)
Instrument	CFX96™ Real-Time PCR System (Bio-Rad)
Performance Characteristics	Analytical specificity: 100% Analytical sensitivity (LoD): [Panel A] NG (<i>Neisseria gonorrhoea</i>): 1 copies/µL CA (<i>Candida albicans</i>): 0.5 copies/µL CT (<i>Chlamydia trachomatis</i>): 1 copies/µL

	MH (<i>Mycoplasma hominis</i>): 5 copies/μL [Panel B] MG (<i>Mycoplasma genitalium</i>): 1 copies/μL HSV-1 (Herpes simplex virus 1): 0.5 copies/μL TV (<i>Trichomonas vaginalis</i>): 5 copies/μL UU (<i>Ureaplasma urealyticum</i>): 1 copies/μL [Panel C] GV (<i>Gardnerella vaginalis</i>): 0.5 copies/μL UP (<i>Ureaplasma parvum</i>): 5 copies/μL HSV-2 (Herpes simplex virus 2): 1 copies/μL TP (<i>Treponema pallidum</i>): 1 copies/μL
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4. INDICATION AND METHOD PRINCIPLE

4.1. Indication

PCG® STIs Fast Real-time PCR Kit is an *in vitro* nucleic acid amplification test to simultaneously detect 12 sexually transmitted pathogens in urine or vaginal swab. Pathogens include: *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV), *Mycoplasma genitalium* (MG), *Gardnerella vaginalis* (GV), *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), *Mycoplasma hominis* (MH), *Candida albicans* (CA), *Treponema pallidum* (TP), Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2).

Indications for STIs testing:

- People have unprotected sexual contact with multiple partners
- Cases of sexually transmitted disease symptoms
- Partners have been infected with sexually transmitted pathogens
- Premarital health screening

4.2. Principle

PCG® STIs Fast Real-time PCR Kit is designed for detecting nucleic acids of 12 sexually transmitted pathogens based on the Real-time PCR technique. Using 3 difference mixes with an effectively optimized thermal cycle to reduce reaction time. In addition, the kit contains the Uracil-DNA Glycosylase (UDG) system and internal control (IC) for contamination prevention and quality control.

5. PROCESS

5.1. PCR reaction mix preparation

- Thaw reagents on ice.
- Invert all reagents well and spindown gently before use.
- Prepare 3 mixes A, B, C depend on the number of samples in the run (including clinic sample, negative control and positive control). The reaction components for 1 sample are shown in the table below:

Components	Mix A	Mix B	Mix C
STI Master Mix	10 μL	10 μL	10 μL
STI-A	5 μL	-	-

STI-B	-	5 µL	-
STI-C	-	-	5 µL

- Vortex and spindown briefly.
- Dispense 15 µL each PCR reaction mix A/B/C into 96 well plate or PCR tube.

5.2. DNA Extraction

DNA extraction from clinical samples should be performed according to manufacturer's instructions for use and recommended using the PCG® MagBead DNA Extraction Kit (Phacogen, Cat. No PR5000050).

5.3. Addition of sample

- Add 5 µL DNA sample/Negative Control/Positive Control to each 96 well plate or PCR tube containing mix A, B, C.
- After sealing with film or cap, spindown briefly.

5.4. Real-time PCR

- Set the 'CFX96™ System' fluorescent channel as below:

Fluorophore	Mix A	Mix B	Mix C
FAM	NG	MG	GV
HEX	CA	HSV-1	UP
Texas Red	CT	TV	HSV-2
Cy5	MH	UU	TP
Quasar 705	IC	IC	IC

- Set the Ramp rate of Real-time PCR device:

Up ramp.	Down ramp.
5°C/sec	Default

- Set the amplification condition as below:

PCR Step	Temperature	Duration (min:sec)	Cycle
Pre-Denaturation	95°C	05 : 00	1
Denaturation	98°C	00 : 10	5
Annealing and Extension	58°C	00 : 20	
Denaturation	98°C	00 : 01	40
Annealing and Extension (Detection)	58°C	00 : 08 (FAM, HEX, Texas Red, Cy5, Quasar 705)	

- Place the plate or tubes into the device according to the declared position and start the Real-time PCR program.

6. DATA ANALYSIS & RESULTS INTERPRETATION

Open the result file, select “Settings→Cq Determination Mode→Regression”.

Confirm “positive result” for Ct ≤39.0 (+) and “negative result” for Ct ≤39.0 (-).

Determining positive pathogen depends on the signals in the mixes. Control samples are valid only if the Ct values comply with the following table:

	Mix	FAM	HEX	Texas Red	Cy5	Quasar 705	Result Interpretation
Sample	A	(+)	(+)	(+)	(+)	(+)	NG, CA, CT, MH (+)
	B	(+)	(+)	(+)	(+)	(+)	MG, HSV-1, TV, UU (+)
	C	(+)	(+)	(+)	(+)	(+)	GV, UP, HSV-2, TP (+)
Negative Control	A, B, C	(-)	(-)	(-)	(-)	(-)	Valid
Positive Control	A, B, C	(+)	(+)	(+)	(+)	(+)	Valid

Note:

- If IC is not detected from sample with target detected, the result is determined as positive. Yet, sequencing is suggested in order to have more accurate result.
- If IC is not detected from sample with target not detected, re-do the test.

7. MATERIAL AND EQUIPMENT REQUIRED NOT PROVIDED WITH THE KIT

Devices	Consumable Material
- CFX96™ Real-Time PCR System - Vortex mixer - Spindown centrifuge - Pipettes - Cooling block - PCR workstation - Laboratory freezers	- Real-time PCR tubes or plates for CFX96 - Pipette tips with filters - Personal protective equipment

8. WARNINGS AND PRECAUTIONS

- The optimum volume of each reaction is 20 µL. Any change in volume will lead to worse results.

- The workflow in the laboratory should be proceed in unidirectional manner from the PCR mix room, the nucleic acid extraction room, and the amplification room, do not go opposite directions. Each area has its own devices and materials.
- Specimens and related items should be handled as a source of infection.
- Always wear protective disposable powder-free gloves when handling. Change gloves if there is any suspicion of cross-contamination or as needed.
- Before and after work, it is necessary to clean working area with PCG[®] DNAzap or equivalent.
- The presence of PCR inhibitors may cause false negative or invalid results.
- The kit must be performed by trained people.
- Process and precautions must be strictly followed to obtain accurate results.
- Pay attention to the expiration date printed on the box and labels of all components.
- Specimens are collected, transported, and stored according to proper procedures and under appropriate conditions. Improper collection, transportation, or storage of samples can give incorrect test results.
- Do not mix components from kits with different lot numbers.
- Use always sterile pipette tips with filters to avoid cross-contamination.

9. EXPLANATION OF SYMBOLS