Chlamydia Trachomatis（CT）Real Time PCR Kit

User Manual

For In Vitro Diagnostic Use Only

IVD

-20 °C

REF SD-0016-02-A+B

For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4; LightCycler®480 Instrument

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1. Intended Use

Chlamydia trachomatis real time PCR kit is used for the detection of Chlamydia trachomatis in genital swabs or urine samples by using real time PCR systems.

Its characteristics:

High sensitivity: lower detection line 5×10^3 copies/ml； LOQ：10^4 ～10^8 copies/ml

(Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.)

High specificity: test result will be positive, only to Chlamydia trachomatis.

Short operating time: 2 and a half hours totally

Good stability: kept for 12 months at –20℃; CV≤5%;

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Chlamydia trachomatis is a small bacterium that cannot grow outside a living cell. In this respect it resembles a virus, but it is actually a very sophisticated organism. It is a natural pathogen to humans. Worldwide, the most important disease caused by Chlamydia trachomatis is trachoma, one of the commonest infectious causes of blindness. In some parts of the developing world, over 90% of the population becomes infected. The organism often causes genital tract infection. In men, Chlamydia trachomatis is the commonest cause of non-gonococcal or non-specific urethritis. In women, the organism may infect both the cervix and the urethra. Epididymitis may complicate the infection in men, whilst in women infection in the upper genital tract, may lead to acute pelvic inflammatory disease (PID).Chlamydia trachomatis real time PCR kit contains a specific ready-to-use system for the detection of the Chlamydia trachomatis by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the chlamydia trachomatis DNA. Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified chlamydia trachomatis DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and genital swabs samples are used for the extraction of the DNA. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). Four quantitation standards are supplied, allows the determination of the gene load.

4. Kit Contents

	Ref.	Cap Color	Type of reagent	Presentation 25rxns
PCR System	1	Orange	DNA Extraction Buffer	2 vials, 1.5ml
	2	Red	CT Reaction Mix	1 vial, 950µl
	3	Blue	PCR Enzyme Mix	1 vial, 12µl
	4	Yellow	Molecular Grade Water	1 vial, 400µl
	5	Orange	Internal control (IC)	1 vial, 30µl
QS ★ System	6	Pink	CT QS1 (5×10^7copies/ml)	1 vial, 20µl
	7	Purple	CT QS2 (5×10^6copies/ml)	1 vial, 20µl
	8	Orange	CT QS3 (5×10^5copies/ml)	1 vial, 20µl
	9	Yellow	CT QS4 (5×10^4copies/ml)	1 vial, 20µl

★QS：Quantitation Standard

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Real time PCR reaction tubes/plates
- Vortex mixer
- Pipets (0.5 µl – 1000 µl)
- Cryo-container
- Disposable gloves, powderless
- Sterile microtubes
- Biohazard waste container
- Refrigerator and freezer
- Sterile filter tips for micro pipets
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. ⚠Warnings and Precaution

Carefully read this instruction before starting the procedure.

- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Avoid aerosols

8. Sample Collection, Storage and transport

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local

regulations for the transport of etiologic agents

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit.

Attention: please thaw the buffer thoroughly and mix the buffer well before use because it contains insoluble particles. You may use your own extraction systems or commercial kit**s.**

9.1.1 Genital swabs sample

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction systems or commercial kit**s.**

1) Wash the genital swabs in 1.0ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

2) Add 1.0ml normal saline and suspend the pellet with vortex vigorously. Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

3) Add 50μl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

4) Incubate the tube for 10 minutes at 100°C.

5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.2 Urine sample

1) Take 1.5 ml sample to a tube, Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

2) Add 1.0ml normal saline then vortex vigorously. Centrifuge the tube at 15000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.

3) Add 50μl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

4) Incubate the tube for 10 minutes at 100°C.

5) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

Attention:

A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.

B. The extraction sample should be used in 3 hours or stored at -20°C for one month.

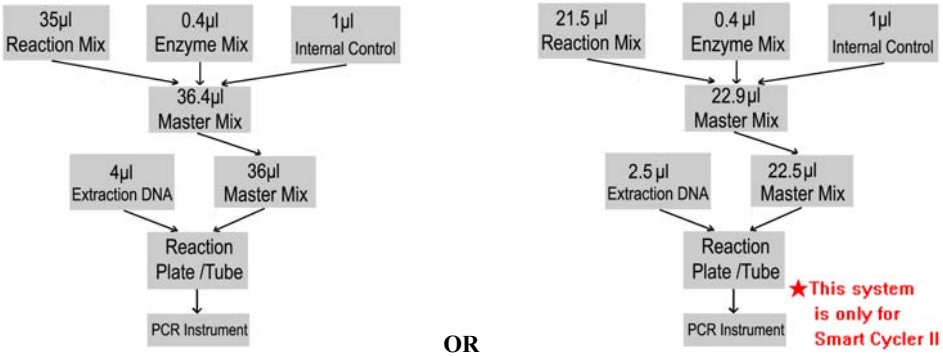
C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal control (IC) allows the user to determine and control the possibility of PCR inhibition.

9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



※ PCR system without HEX/VIC/JOE channel may be treated with 1μl Molecular Grade Water instead of 1μl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.

2) Pipet **36μl (22.5μl for SmartCyclerII)** Master Mix with micropipets of sterile filter tips to each of the *Real time* PCR reaction plate/tubes. Separately add **4μl(2.5μl for SmartCyclerII)** DNA sample, QS1,QS2,QS3,QS4 and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.

3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.

4) Perform the following protocol in the instrument:

37°C for **2 min,1 cycle;94°C** for **2 min,1 cycle;93°C** for **15 sec,60°C** for **60 sec,40 cycles.**

Fluorescence is measured at 60°C； FAM and HEX/VIC/JOE channels should be chosen.

5) ⚠If you use ABI Prism® system,please choose **"none"** as **passive reference** and **quencher.**

10. Threshold setting: just above the maximum level of molecular grade water.

11.Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12.Quality control:

Channel Control	Ct value	
	FAM (Target Nucleic Acid)	HEX/VIC/JOE (IC)
Molecular Grade Water	UNDET	25~35
QS1/QS2/QS3/QS4	≤38，and Correlation coefficient of QS curve≤－0.98	——

13. Data Analysis and Interpretation： The following results are possible:

1) The Ct value in channel FAM shows ≤38. **The result is positive: The sample contains CT DNA. Quantitative value of samples is automatically reported according to the standard curve.**

Quantitative Value	**Data Analysis and Suggestion**
$<5\times10^3$copies/ml	CT DNA Positive; its concentration lower than 5×10^3copies/ml
$5\times10^3\sim10^4$ copies/ml	CT DNA Positive; the quantitative value for recommendation only
$10^4\sim10^8$ copies/ml	CT DNA Positive; the quantitative value is valid
$>10^8$ copies/ml	1)CT DNA Positive but the quantitative value for recommendation only 2)Re-test the sample after dilute the sample by several times, making the quantitative value within $10^4\sim10^8$ copies/ml

2) The Ct value in channel FAM shows 38~40, please repeat again. **If the result still shows 38~40,it can be considered negative.**

3) In channel FAM no signal is detected, at the same time, a HEX/VIC/JOE signal from the Internal Control appears. **The sample does not contain any CT DNA. It can be considered negative.**

4) Neither in channel FAM nor in channel HEX/VIC/JOE is a signal detected. **A diagnostic statement can not be made.** Inhibition of the PCR reaction.

For further questions or problems,please contact our technical support at **trade@liferiver.com.cn**