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CT&NG Real Time PCR Kit User Manual
For In Vitro Diagnostic Use Only

REF **SD-0025-01**

Σ 25 −20 °C IVD

For use with LightCycler1.0/2.0 Instrument

Shanghai ZJ Bio-Tech Co., Ltd.
www.liferiver.com.cn Tel: +86-21-34680596
trade@liferiver.com.cn Fax: +86-21-34680595
2nd floor,No.15 Building,No.188 Xinjunhuan road,
PuJiang Hi-tech Park Shanghai China

1. Intended Use
CT&NG real time PCR kit is used for the detection of Chlamydia trachomatis and Neisseria Gonorrhoeae in genital swabs and urine specimens by using real time PCR systems.

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
Neisseria gonorrhoeae and Chlamydia trachomatis are recognized as two of the most prevalent sexually transmitted bacterial infections. Worldwide, there is an estimated annual incidence of 25 million cases of gonorrhea and 50 million cases of chlamydia. In an effort to prevent the spread of these diseases, increased attention is being focused on early diagnosis and treatment of symptomatic or asymptomatic infected individuals. In men, C. trachomatis causes 40 to 50% of cases of nongonococcal urethritis, making it one of the most common sexually transmitted diseases (STD) in heterosexual males. In women, chlamydia is a major cause of pelvic inflammatory disease leading to infertility and ectopic pregnancy and can result in conjunctivitis and pneumonia in newborn infants exposed during passage through an infected birth canal.Symptoms and complications of gonorrhea are similar to those of chlamydia, and a substantial proportion of infected individuals,especially women, are asymptomatic. For both organisms,asymptomatic persons serve as a reservoir of infection,and since coinfection is common, symptoms may overlap, making clinical diagnosis difficult.

CT&NG real time PCR kit contains a specific ready-to-use system for the detection of the CT&NG 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 530nm** with the fluorescent quencher BHQ1. Detection of amplified Neisseria gonorrhoeae DNA fragment is performed in fluorimeter **channel 560nm** 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 **channel 610nm** fluorescence of the internal control (IC).An external positive control (1×10^7copies/ml) contained, allow the determination of the gene load.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	CT &NG Reaction Mix	1 vial, 480µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control	1 vial, 30µl
6	CT&NG Positive Control(1×10^7copies/ml)	1 vial, 30µl

Analysis sensitivity: 1×10^4 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.

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

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.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- 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. It's better to use commercial kits for nucleic acid extraction.

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.

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.

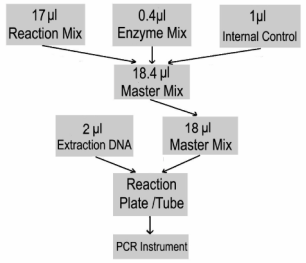
9.1.2 Urine sample
1) Take 1.5 ml sample to a tube, Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 1.0ml normal saline then vortex vigorously. Centrifuge the tube at 15000rpm for 5 minutes, carefully remove and 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, for the vapor will volatilize into the air and may cause contamination in case 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 DNA extraction, please comply with manufacturer's instructions.

9.2 Internal Control and Positive 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.
Add the internal control (IC) 1µl/rxn and the result will be shown in the 610nm.

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



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 **18µl** Master Mix with micropipets of sterile filter tips to each of the *Real time* PCR reaction plate/tubes. Separately add **2µl** DNA sample, positive 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 2min	1cycle
94°C for 2min	1cycle
93°C for 5sec, 60°C for 30sec (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
530nm	CT
560nm	NG
610nm	IC

10. Threshold setting: Choose **Arithmetic** as back ground and **none** as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

11.Quality control:
Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Crossing point value		
	530nm	560nm	610nm
Molecular Grade Water	Blank	Blank	25~35
Positive Control(qualitative assay)	≤35	≤35	——

12. Data Analysis and Interpretation
The following sample results are possible:

	Crossing point value		Result Analysis
	530nm/560nm channel	610nm channel	
1#	Blank	25~35	Below the detection limit or negative
2#	⩽38	——	Channel 530nm: CT positive Channel 610nm: NG positive
3#	38～40	25~35	Re-test; If it is still 38~40, report as 1#
4#	Blank	Blank	PCR Inhibition; No diagnosis can be concluded.

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