Chlamydia Trachomatis Nucleic Acid Detection Kit Manual (Fluorescent PCR)

【Product name】

Chlamydia Trachomatis Nucleic Acid Detection Kit (Fluorescent PCR)

【Packaging specification】

24 tests/box

【Intended use】

The kit is used for in vitro qualitative nucleic acid detection of chlamydia trachomatis (CT) in male urethral samples and female urethral and cervical secretion samples.

Chlamydia trachomatis is a kind of prokaryotic microorganism that can pass through the filter, parasitize strictly in eukaryotic cells, and has a unique development cycle. It can cause trachoma, inclusion body capsulitis, urogenital tract infection and lymphogranuloma of venereal disease. Clinically, males are mainly characterized by nongonococcal urethritis, prostatitis, epididymitis, proctitis and infertility. In women, it is cervicitis, and cervical hyperemia erosion with purulent secretion, salpingitis, ectopic pregnancy, infertility, abortion, etc. There are three routes of transmission of chlamydia trachomatis: sexual contact transmission, vertical transmission from mother to child, and indirect contact transmission. Chlamydia infection has become a serious social problem. On one hand, chlamydia has become the most common pathogen in sexually transmitted diseases. On the other hand, it can cause serious persistent infection.

【Detection principle】

The kit adopts polymerase chain reaction (PCR) and fluorescence labeling probe technology, namely adding fluorescent groups into the PCR reaction system to monitor the whole PCR process in real time by utilizing fluorescence signal accumulation. It can rapidly detects highly conservative specific nucleic acid sequences (nucleic acid sequences of encoding omp1 membrane protein) of chlamydia trachomatis in clinical samples by fluorescence PCR technology so as to judge the existence of chlamydia trachomatis.

【Kit contents】

Contents		Specification and quantity	Ingredients
1	DNA Extraction Solution	1200μL x 1 vial	Chelex 100, Tris HC1, NaOH, Triton-100, NP-40, EDTA
2	CT-PCR Reaction Solution	1056μL x 1 vial	Probes, primers, Dntp, buffer system
3	DNA Polymerase	24μL x 1 vial	Taq NDA polymerase, UNG enzyme, suc Ⅱ plasmid
4	Positive Control	200μL x 1 vial	Plasmids containing target genes
5	Weak Positive Control	200μL x 1 vial	Plasmids containing target genes
6	Negative Control	200μL x 1 vial	Nucleic acid dilute solution

Note: The positive control is plasmid containing the target gene, which is from the original strain of the American Strain Collection Center (ATCC).

【Storage and validity】

Kit should be kept at -18℃ and it is valid for 6 months. Keep at 2-8℃ after opening and use within 1 week. Avoid repeated freezing and thawing (less than 5 times).

Transport the kit at low temperature. Use the ice packs for long distance transportation. Refer to the label for manufacture date and validity date.

【Compatible instruments】

TIB-8600, ABI 7500, STRATAGENE Mx3000P fluorescent PCR instrument.

【Sample requirements】

1. Specimen types: male urethral samples, female urethral and cervical secretion samples.

2. Recommended sampling swab: sterile swab. The swab head is made of polyester, fiber, nylon and other materials. The swab handle is made of plastic.

3. Specimen collection:

3.1 Male urethral sample: collect the urethral secretion or insert a small swab into urethra about 2-4cm, rotate gently to obtain secretion, and seal the swab into the tube for inspection.

3.2 Female urethral sample: clean the urethra orifice with sterile normal saline, insert sterile swab into urethra about 2cm and rotate to obtain secretion samples, and seal the swab into the tube for inspection.

3.3 Female genital tract sample: wipe off excessive secretion in the genital tract with sterile physiological saline, place sterile polyester swab at the lower 1/3 of the genital tract, gently rotate to obtain secretion, and seal the swab into the tube for inspection.

4. Specimen preservation and transportation: specimens can be sent for inspection immediately, and shall not be stored for more than 6 days at 4-25℃. Non-liquid specimens can be stored for 6 months at -20℃. Ice bags shall be used for long distance transportation.

【Procedure】 (Please read this operating procedure carefully before use)

1. Reagent preparation (reagent preparation area)

1.1 Prepare DNA extraction solution

1.2 Confirm the number of reaction tubes n (n = sample number + negative control+ positive control + weak positive control) to be carried out. Pipet n×44μL CT-PCR reaction solution and n×1μL DNA polymerase into a centrifuge tube, vortex and spin down. Aliquot into n x PCR reaction tubes by 45μL of each tube. After covering the tube cover, transfer to the sample addition area, and place in a refrigerator at 4℃.

1.3 Transfer the quality controls and reference controls to the sample processing area and place them in a refrigerator at 4℃.

2. Sample processing (sample processing area)

2.1 Sample processing steps:

2.1.1 Add 1mL of sterilized normal saline to the swab tube, vortex fully and squeeze dry the swab.

2.1.2 Transfer all the liquid treated in step 2.1.1 to a 1.5mL centrifuge tube (if there is too much secretion, transfer 200μL only), and centrifuge at 10000 rpm for 5 minutes.

2.1.3 Discard the supernatant and add 1mL of sterilized normal saline to the precipitate and vortex fully. Centrifuge at 10000rpm for 5 minutes.

2.1.4 Discard the supernatant and add 50μL of DNA extraction to the precipitate and vortex fully.

(the extraction solution contains water-insoluble substances, vortex fully before pipetting) Treat at 100℃ constant-temperature for 10 minutes.

2.1.5 Centrifuge at 10000 rpm for 5 minutes, and keep the supernatant for PCR reaction.

2.2 Treatment of negative control

2.2.1 Spin down the negative control and pipet 50μL to 1.5mL centrifuge tube. Add 50μL DNA extraction and fully vortex. Treat at 100℃ constant-temperature for 10 minutes.

2.2.2 Treat at 4℃ and let stand for 10-20min, then centrifuge at 10000 rpm for 5 minutes. Keep the supernatant for PCR reaction.

2.3 Treatment of positive control: (same as negative control)

2.4 Treatment of weak positive control: (same as negative control)

3. PCR reaction

3.1 Sample adding (sample processing area or sample adding area)

Add 5μL of sample, negative control, positive control, weak positive control supernatant to the prepared PCR reaction tubes respectively, or add 5μL quantitative reference control directly. Spin down immediately after covering the tube tightly.

3.2 PCR amplification (Detection area)

Place the PCR tubes into the PCR instrument, edit the sample information and amplify as per below sequence:

Cycle Parameters:

Stage 1	37℃	---- 2 min
Stage 2	94℃	---- 2 min
Stage 3		
40 cycles of		
	94℃	---- 15 sec
	55℃	---- 45 sec

CT Fluorescein detection: FAM

Internal control fluorescein: HEX (use JOE channel if HEX is not available on the instrument)

Reaction volume: 50μL

Fluorescent signal collection: stage 3, 55℃ --- 45 sec

Collect at the end.

CT-PCR reaction system includes CT detection and internal control.

【Positive judgement】

Using the instrument matching software to implement automatically analysis, and obtain Ct values for all the samples and controls.

1	Specimen (FAM) Ct value ≤ 33 with nice log amplification curve	Positive	
2	Specimen (FAM) Ct value = 40, or "No Ct" (Mx3000P) or "Undet" (ABI 7500), Internal Control (HEX) Ct value ＜ 30 with nice log amplification curve.	Negative (below LOQ)	
3	Specimen (FAM) 33 ＜Ct value ＜ 40	Vague result area, should be tested two more times	Retest twice, Ct value=40, negative
			Retest twice, at least one Ct value ＜ 40 with nice log amplification curve, suspected positive, collect the specimen and test one more time or other detection methods suggested

【Result analysis condition setting】

1. ABI 7500 baseline setting: take the fluorescent signal line between cycle 2 and the sample cycle number 3 cycles before threshold is reached as the baseline. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 40 or "Undet"
2. STRATAGENE Mx3000P baseline setting: select the fluorescence signal when "Adaptive baseline" is set. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 40 or "No Ct"

【Quality control standards】

Positive and negative control should meet the following standards at the same time, otherwise the test is invalid.

1. Negative control, CT (FAM) Ct value = 40 or "No Ct" (Mx 3000p) or "Undet" (ABI 7500), internal control (HEX) Ct value ＜40 with nice log amplification curve.
2. Positive control, CT (FAM) has nice log amplification curve. Quantitative reference value is between 1.0×10^6copies/mL to 1.0×10^7copies/mL.
3. Weak positive control, CT (FAM) has nice log amplification curve. Quantitative reference value is between 1.0×10^3copies/mL to 1.0×10^4copies/mL.

【Results Interpretation】

1. CT negative (lower than limit of quantitation): CT FAM Ct value = 40 or "No Ct" (Mx 3000p) or "Undet" (ABI 7500), internal control (HEX) Ct value ＜40 with nice log amplification curve.
2. CT positive: CT (FAM) Ct value ≤33 with nice log amplification curve, internal control (HEX) Ct value ≤40.

3. Invalid results, retest is needed. CT FAM Ct sample = 40 or "No Ct" (Mx 3000p) or "Undet" (ABI 7500), internal control (HEX) Ct value = 40 or "No Ct" (Mx 3000p) or "Undet" (ABI 7500)

4. For samples with vague results: FAM 33 ＜ Ct sample ＜ 40, retests are suggested.

Note: Test result is for clinical reference only, and should not be taken as the only evidence to diagnose CT infection. Negative result only means that the DNA content is lower than the LOQ of this kit and should not exclude the possibility of CT infection. Further confirmation combining other clinical data is highly suggested for making a definite diagnosis when the result is positive.

【Detection Limitation】

This kit is suitable for clinical specimens detection, but the results are affected by the instruments and operation. Therefore, the results are for reference of clinical diagnosis only, and not the only criterion to confirm or exclude disease cases.

【Kit Performance】

1. The detection lower limit for this kit is 5.0×10^{2}copies/mL, with a linear range between 5.0×10^{8}copies/mL and 5.0×10^{2}copies/mL.

2. It is verified that this kit will not cross-react with other common clinical pathogens as below.

No.	Pathogens	Origin	Concentration	Interference
1	Candida albicans	CMCC	10^{5}-10^{6} bacterium/mL	No interference
2	Staphylococcus aureus	ATCC	10^{5}-10^{6} bacterium/mL	No interference
3	Group B strep	ATCC	10^{5}-10^{6} bacterium/mL	No interference
4	Chlamydia psittaci	ATCC	10^{5}-10^{6} bacterium/mL	No interference
5	Chlamydia pneumoniae	CMCC	10^{5}-10^{6} bacterium/mL	No interference
6	Ureaplasma urealyticum	CMCC	10^{5}-10^{6} bacterium/mL	No interference
7	Mycoplasma genitalium	CMCC	10^{5}-10^{6} bacterium/mL	No interference
8	Mycoplasma hominis	CMCC	10^{5}-10^{6} bacterium/mL	No interference
9	Escherichia coli	CMCC	10^{5}-10^{6} bacterium/mL	No interference
10	Staphylococcus epidermidis	CMCC	10^{5}-10^{6} bacterium/mL	No interference
11	Herpes Simplex Virus Ⅰ	ATCC	10^{5}-10^{6} bacterium/mL	No interference
12	Herpes Simplex Virus Ⅱ	ATCC	10^{5}-10^{6} bacterium/mL	No interference
13	Neisseria gonorrhoeae	CMCC	10^{5}-10^{6} bacterium/mL	No interference
14	Human papilloma virus	ATCC	10^{5}-10^{6} bacterium/mL	No interference
15	Cytomegalovirus	ATCC	10^{5}-10^{6} bacterium/mL	No interference

3. The CV value within same batch and between different batches are both lower than 10%.

4. In the detection of clinical specimens of Chlamydia Trachomatis, the coincidence rate of this kit compared with competing kit (SFDA approved) is above 95%, which meets the requirements of clinical application.

5. Experiments prove that normal concentrations of human interfering substances such as blood, urine, mucus and normal concentrations of external drugs, mold preparation, metronidazole and miconazole nitrate will not affect the experimental results.

【Warnings and Precaution】

1. For in vitro diagnosis use only.
2. Read this manual in detail before the assay, and the assay should be carried out by skilled personnel.
3. Use latex gloves or thin film gloves when handling the PCR tubes.
4. Avoid unnecessary repeated freezing and thawing and keep the PCR solution away from lights since there are enzyme and fluorescent probes in PCR reaction solution.
5. Thoroughly thaw the reagents and spin them down briefly before using.
6. Sterilize centrifuge tubes and pipet tips in high temperature and high pressure before being used.
7. Processing and handling of clinical specimens should be carried out in a biosafety cabinet.
8. After being spin down, the PCR tubes should avoid vortex when being loaded on the PCR instrument.
9. Avoid touching the precipitation when aspirate the template.
10. Paraffin is suggested for sealing and cover the tube caps tightly after sample adding.
11. Dispose the PCR tubes in sealing airtight plastic bags as biohazard waste after the PCR instrument cool down at room temperature.
12. Dispose the pipet tips into the 10% sodium hypochlorite wasted solution vat and sterilize with other wastes.
13. Sterilize the biohazard safety cabinet by UV lights. After the experiment, clean the biohazard cabinet and pipets with 10% pasteurization, then use 75% ethyl alcohol for cleaning after 10 min.
14. Do not mix-use the reagents from different batches. Use this kit before its expiration.
15. All components of this kit may be toxic, avoid entering mouth.

【Manufacturer】

Name of registered manufacturer: Triplex International Biosciences (China) Co., Ltd.
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