Treponema Pallidum (TP) Nucleic Acid Quantitative Diagnostic Kit Manual (Fluorescent PCR Method)

【Product Name】

Generic name：Treponema Pallidum (TP) Polymerase Chain Reaction (PCR) Fluorescence Diagnostic Kit

【Packaging Specification】

24 tests/box

【Intended Use】

This kit is suitable for in vitro quantitative detection of treponema pallidum (TP) nucleic acid in reproductive urinary tract secretion swab, serum and placental tissue samples. It can be used for auxiliary diagnosis and efficacy monitoring of treponema pallidum (TP) infection. The results are only for clinical reference, and should not be taken as the only basis for clinical diagnosis.

【Detection Principle】

The kit combines PCR technology with fluorescent probe technique to rapidly detect the specific nucleic acid sequence of TP in the sample to determine the presence of TP.

【Kit Contents】

Item	Contents		Spec and Quantity	Ingredients
1	DNA Extraction Buffer		1200μl/vial × 1vial	chelex 100、Tris HCl、NaOH、Triton-100、NP-40、EDTA
2	DNA Concentration Solution		1200μl/vial × 2 vials	PEG6000、NaCl
3	TP-PCR Reaction Solution		1056μl/vial × 1 vial	Buffer、probe、primer、dNTP、$MgCl_2$、
4	Taq Enzyme		24μl/vial ×1 vial	Taq DNA Polymerase、UNG Enzyme
5	Positive Control		200μl	Plasmids containing target gene
6	Weakly Positive Control		200μl	Plasmids containing target gene
7	Negative Control		200μl	Tris-HCl、EDTA
8	Quantitative Reference	1.0×10^4copies/ml	20μl	Plasmids containing target gene
		1.0×10^5copies/ml	20μl	Plasmids containing target gene
		1.0×10^6copies/ml	20μl	Plasmids containing target gene
		1.0×10^7copies/ml	20μl	Plasmids containing target gene

【Storage and validity】

Storage：Stored at -20±50 ℃, the kit is valid for 6 months. After opening, stored at 4 ℃ and use them within 7 days. Avoid repeated freezing and thawing. (Less than 5 times)

Transportation：Low temperature transportation and for long distance transportation, use the foam box equipped with ice bags

【Compatible Instruments】

ABI 7500、STRATAGENE Mx3000P fluorescence PCR instruments etc.

【Sample Requirements】

1. Sample types：Reproductive urinary tract secretion, serum and placental tissue samples etc.

2. Sample collection：

2.1 Male urinary tract sample： Carefully insert the sterile swab into the urinary tract about 2cm and gently rotate. Stay for a few seconds to obtain the secretion sample and put the swab back into the sterile swab sleeve and send to testing spot in a closed condition.

2.2 Female urinary tract sample：Clean the meatus urinarius with sterile saline solution first. Carefully insert the sterile swab into the urinary tract about 2cm and gently rotate to obtain the secretion sample and put the swab back into the sterile swab sleeve and send to testing spot in a closed condition.
2.3 Female genital tract sample: Wipe off the excessive secretion with sterile saline solution of cervix. Carefully insert the sterile swab into the cervix and stay for 5 seconds to obtain the mucosa secretion sample and put the swab back into the sterile swab sleeve.
2.4 Serum sample：Extract 2ml venous blood with disposable sterile syringes and transfer into sterile dry glass tube. Keep the tube at room temperature (22 ~ 25 ℃) for 30 ~ 60 minutes. The blood specimens can spontaneously congeal and precipitate serum or centrifuge the blood specimens at 1500 RPM for 5 minutes. Pipet the upper serum and transfer into 1.5ml sterilized centrifuge tube.
2.5 Placental tissue samples：Take appropriate amount of placental tissue from childbirth or induced labor into sterile glass tub and send to testing spot in a closed condition.

3. Sample storage and transport：Samples can be used for immediate detection or can be kept at -20℃ for detection. Storage life is 6 months. Ice packs should be used during sample transportation..

【Procedure】

1. Reagent Preparation

1.1 Take out the DNA Extraction Buffer for preparation.
1.2 Calculate the reaction number n (n = specimen number + negative control + positive control + weakly positive control + 4 quantitative reference). Take out the TP-PCR Reaction Solution. n×44μl TP-PCR reaction solution and n×1μl Taq DNA Polymerase were added into a centrifuge tube, vortexed briefly, spin down briefly, aliquoted into N tubes with 45μl/tub. After covered with covers, all the PCR reaction tubes are moved to sample adding area, and are stored at 4 degrees and away from light for further use.
1.3 Transfer the negative control, positive control, weakly positive control and quantitative reference into 4 ℃ refrigerator as well.

2. Sample Processing

2.1 Genital and urinary tract samples

a. Add 1ml sterilized saline solution into the tube and vortex fully, and then squeeze the swab
b. Transfer all the liquid from the above step into 1.5ml centrifuge tube（pipet only 200ul if too much secreta）and centrifuge at 12000rpm for 5 minutes
c. Discard the supernatant and add 1ml sterilized saline solution, vortex fully and centrifuge at 12000rpm for 5 minutes.
d. Discard the supernatant and add 50μL DNA extraction solution and vortex.（Mix well before pipetting since there are some water insoluble granular materials in the DNA extraction solution）, 100℃ constant temperature processing for 10±1 minutes.
e. Centrifuge at 12000rpm for 5 minutes and use the supernatant for PCR reaction.

2.2 Serum samples

a. Pipet 100μL serum and 100μL DNA concentration solution and vortex for 5 seconds
b. Centrifuge at 12000rpm for 10 minutes
c. Discard the supernatant as much as possible without stirring or touching the sediments.
d. Discard the supernatant and add 50μL DNA extraction solution and vortex.（Mix well before pipetting since there are some water insoluble granular materials in the DNA extraction solution）, 100℃ constant temperature processing for 10±1 minutes.
e. Centrifuge at 12000rpm for 5 minutes and use the supernatant for PCR reaction.

2.3 Placental tissue samples

a. Clean the blood with appropriate amount of sterilized saline solution
b. Take 50mg tissue and add 1ml sterilized saline solution. Use the homogenizer to grind them into tissue homogenate. Transfer into 1.5ml centrifuge tube and centrifuge at 12000rpm for 5 minutes.
c. Discard the supernatant and add 50μL DNA extraction solution and vortex.（Mix well before pipetting since there are some water insoluble granular materials in the DNA extraction solution）, 100℃ constant temperature processing for 10±1 minutes.
d. Centrifuge at 12000rpm for 5 minutes and use the supernatant for PCR reaction.

2.4 Negative control sample processing

Briefly spin down the negative control. Pipet 50μL into 1.5ml centrifuge tube and add 50μL DNA extraction solution.. (Mix well before pipetting since there are some water insoluble granular materials in the DNA extraction solution), 100℃ constant temperature processing for 10±1 minutes. Centrifuge at 12000rpm for 5 minutes and use the supernatant for PCR reaction.

2.5 Positive control sample processing（same process as negative control）

2.6 Weakly positive control sample processing（same process as negative control）

2.7 Quantitative reference：Centrifuge at 12 000rpm for a few seconds and then use for PCR reaction

3. PCR Reaction

3.1 sample adding

Pipet 5μL supernatant, specimen solution, positive control, negative control, weakly positive control and quantitative reference into the PCR reaction tubes respectively. Seal the covers tightly and briefly spin down the PCR reaction tubes.

3.2 PCR amplification

Put the PCR reaction tubes into the PCR instrument, input the specimen info and then proceed with amplification according to temperature cycle parameters below.

Cycle Parameters: Stage 1 37 ℃ ----2min； Stage 2 94 ℃ ----2min； 40 cycles Stage 3 94℃----15sec 55℃---- 45sec	TP detection fluorescein: FAM Reaction Volume : 50μl Fluorescent signal collection: Stage 3：55℃ ----45sec

【Reference Value】

Using the instrument matching software to implement automatically analysis, and obtain Ct value (FAM) and C value for all the samples and controls.

1	TP（TP-FAM）Ct =40 or “No Ct		TP DNA content is lower than the LOQ
2	TP（TP-FAM）Ct <40, with nice log amplification curve.	C < 5.00E+002	TP DNA content = C gene copies（For reference only, retest is suggested. Positive result is determined if with nice log amplification curve）
		5.00E+002 ≤C ≤ 5.00E+008	TP DNA content = C gene copies
		C > 5.00E+008	TP DNA content > 5.0×10^{8}copies/ml. For quantitative purpose, dilute the sample into the linear range and retest.

【Results Interpretation】

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】

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

1. Negative control, TP (FAM) Ct value = 40 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500)

2 Positive control, 18≤TP (FAM) Ct value ≤24 with nice log amplification curve.

3 Weakly positive control, 27≤TP (FAM) Ct value ≤33 with nice log amplification curve.

【Results Interpretation】

1. TP Ct value =40 or "No Ct" (Mx3000P) or "Undet." (ABI 7500), which indicates TP DNA content is lower than the LOQ of this kit.
2. TP Ct value < 40 with nice log amplification curve, analyze as per below:
2.1 Sample C value < 5.00E+002，TP DNA content = C gene copies（For reference only, retest is suggested. Positive result is determined if with nice log amplification curve）
2.2 5.00E+002 ≤ sample C value ≤ 5.00E+008， TP DNA content = C gene copies
2.3 TP DNA content > 5.0×10^8copies/ml. For quantitative purpose, dilute the sample into the linear range and retest.

【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】

The detection lower limit for this kit is 5.0×10^2 copies/ ml with a linear range between 5.0×10^8copies/ ml and 5.0×10^2 copies/ ml.
It is verified that this kit will not cross-react with other clinical regular pathogens.
The CV value within same batch and between different batches are both lower than 10%.

【Warnings and Precaution】

1. This product is for in vitro diagnosis purpose only.
2. Please read the full text of the instruction carefully before the experiment. Test 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
5. Thoroughly thaw the reagents and spin them down briefly before using.
6. The PCR reaction tubes, centrifuge tubes and pipet-tips used for PCR reaction preparation should be autoclaved and used as disposable.
7. Specimen handling should be carried out in biosafety cabinets.
8. After being spin down, the PCR tubes should avoid vortex when being loaded on the PCR instrument.
9. Discard the supernatant as much as possible without touching the sediments.
10. Paraffin is suggested for sealing.
11. After amplification, when the temperature of PCR instrument reduces to room temperature, the reaction tubes should be unloaded and sealed in a special plastic bag and disposed as medical wastes.
12. The pipet-tips used in the experiment should be dismounted directly into the waste tank containing 10% sodium hypochlorite and discarded after sterilized together with other waste materials.
13. Before every experiment, sterile the workbench and pipette with ultraviolet light. After the experiment, sterile the workbench and pipette with 10% chloric acid and clean with 70% alcohol after 10 minutes.
14. Do not interchangeably use reagents of different batches and use the kit within the validity period.

【Manufacturer】

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