Herpes Simplex Virus Ⅱ (HSV-Ⅱ) Nucleic Acid Detection Kit Manual
(Fluorescent PCR)

【Product name】

Herpes Simplex Virus Ⅱ (HSV-Ⅱ) Nucleic Acid Detection Kit (Fluorescent PCR)

【Packaging specification】

24 tests/box

【Intended use】

The kit is suitable for qualitative nucleic acid detection on herpes simplex virus type Ⅱ (HSV-Ⅱ) from herpes ulcer scraping samples and genitourinary tract secretion samples. Human herpes simplex virus is divided into two types, namely herpes simplex virus type Ⅰ (HSV-I) and herpes simplex virus type Ⅱ (HSV-Ⅱ). HSV-Ⅱ belongs to α subfamily of the human herpesviridae in virus taxonomy. The virus is spherical and is DNA virus. It is also known as genital herpes, which mostly infects the lower part of the waist, mainly genital infection. HSV infection is very common in the global population, and human is the only host. Infection is caused mainly through close contact between people and susceptible persons. It can cause herpetic dermatitis, genital herpes, fetal intrauterine infection, fetal abortion, premature delivery, stillbirth and malformation. It can also infect newborns. HSV-I and HSV-Ⅱ are easily to cross infection.

【Detection principle】

The kit adopts polymerase chain reaction (PCR) and fluorescence labeling probe technology, which can rapidly detects the specific nucleic acid sequences of HSV-Ⅱ in clinical samples so as to judge the existence of HSV-Ⅱ.

【Kit contents】

No.	Contents	Specification and quantity	Ingredients
1	Nucleic Acid Extraction Solution	1200μL x 1 vial	Chelex 100, Tris HC1, NaOH, Triton-100, NP-40, EDTA
2	HSV-Ⅱ PCR Reaction Solution	1056μL x 1 vial	Probes, primers, dNTP, $MgCl_2$, buffer system
3	DNA Polymerase	24μL x 1 vial	Taq NDA polymerase, UNG enzyme,
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	Tris HC1, EDTA

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 -20℃ and it is valid for 6 months. Keep at 4℃ after opening and use within 1 week. Avoid repeated freezing and thawing (less than 5 times). Refer to the label for manufacture date and validity date.

Transport the kit at low temperature. Use the ice packs for long distance transportation.

【Compatible instruments】

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

【Sample requirements】

1. Specimen types: herpes ulcer scraping samples or genitourinary tract secretion swab.

2. Specimen collection:

2.1 Use a scraper to scrape the exfoliated cells from the ulcer parts and seal into the tube for inspection.

2.2 Male urethral sample: insert sterile swab into urethra about 2cm and rotate to obtain secretion samples, and seal the swab into the tube for inspection.

2.3 Female urethral sample: clean the urethral 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.

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

3. 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.

Notes: External use (ointment, spray, suppository, lotion, etc.) is prohibited within 24 hours before sample collection.

【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+ strong positive control + weak positive control) to be carried out. Pipet n×44μL HSV-Ⅱ PCR reaction solution and n×1μL DNA polymerase into a centrifuge tube, vortex and spin down. Aliquot 45μL into each PCR reaction tube. After covering the tube cover, transfer to the sample addition area, and place in a refrigerator at 4℃.

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

2. Sample processing (sample processing area)

2.1 Genitourinary tract secretion swabs

2.1.1 Add 1mL sterilized normal saline to the tube, vortex fully for 1 min 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. 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 Urethral orifice or herpes ulcer scraping samples
2.2.1 Add 1mL of sterilized normal saline to the glass tube, vortex fully to elute the cells.
2.2.2 Transfer all the liquid treated in step 2.2.1 to a 1.5mL centrifuge tube and centrifuge at 10000 rpm for 5 minutes.
2.2.3 Discard the supernatant and add 1mL of sterilized normal saline to the precipitate and vortex fully. Centrifuge at 10000rpm for 5 minutes.
2.2.4 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.
2.2.5 Centrifuge at 10000 rpm for 5 minutes, and keep the supernatant for PCR reaction.

2.3 Treatment of negative control
2.3.1 Spin down the negative control and pipet 50μL into 1.5mL centrifuge tube. Add 50μL DNA extraction and vortex fully. (the extraction solution contains water-insoluble substances, vortex fully before pipetting) Treat at 100℃ constant-temperature for 10 minutes.
2.3.2 Centrifuge at 13000rpm for 5 min and keep the supernatant for PCR reaction.
2.4 Treatment of positive control: (same as negative control)
2.5 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 processed sample, negative control, positive control, weak positive control supernatant to the prepared PCR reaction tubes respectively. 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

HSV-Ⅱ 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.
HSV-Ⅱ reaction system includes HSV-Ⅱ detection and internal control.

【Positive judgement】

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

<table>
<tr><td>1</td><td>HSV-Ⅱ (HSV-ⅡFAM) Ct value ≤ 36 with nice log amplification curve</td><td colspan="2">HSV-Ⅱ Positive</td></tr>
<tr><td>2</td><td>HSV-Ⅱ (HSV-ⅡFAM) Ct value = 40, or “No Ct” (Mx3000P) or “Undet” (ABI 7500), Internal Control (HEX) Ct value ＜ 40 with nice log amplification curve.</td><td colspan="2">HSV-Ⅱ Negative (concentration of HSV-Ⅱ DNA is below LOQ)</td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">36＜Ct value ＜ 40</td><td rowspan="2">Vague result area, should be tested two more times</td><td>Retest twice, Ct value=40, HSV-Ⅱ negative</td></tr>
<tr><td>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</td></tr>
</table>

【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, HSV-Ⅱ(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, HSV-Ⅱ (FAM) has nice log amplification curve. 18≤Ct value≤24.
3. Weak positive control, HSV-Ⅱ (FAM) has nice log amplification curve. 27≤Ct value≤33.

【Results Interpretation】

1. HSV-Ⅱnegative: HSV-Ⅱ (HSV-ⅡFAM) Ct value = 40 or “No Ct” (Mx 3000p) or “Undet”

(ABI 7500), internal control (HEX) Ct value ＜40 with nice log amplification curve, it indicates concentration of HSV-Ⅱ DNA is below LOQ.

2. HSV-Ⅱ positive: HSV-Ⅱ (HSV-Ⅱ FAM) Ct value ≤36 with nice log amplification curve, internal control (HEX) Ct value ≤40.
3. Invalid results, HSV-Ⅱ (HSV-Ⅱ FAM) Ct value = 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).

【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 5.0×10^2copies/mL, with a linear range between 5.0×10^8copies/mL and 5.0×10^2copies/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	EB virus	ATCC	10^5-10^6 bacterium/mL	No interference
2	Herpes zoster virus	ATCC	10^5-10^6 bacterium/mL	No interference
3	Cytomegalovirus	ATCC	10^5-10^6 bacterium/mL	No interference
4	Human herpes virus type 6	ATCC	10^5-10^6 bacterium/mL	No interference
5	Human herpes virus type 5	CMCC	10^5-10^6 bacterium/mL	No interference
6	Human herpes virus type 8	CMCC	10^5-10^6 bacterium/mL	No interference
7	Ureaplasma urealyticum	CMCC	10^5-10^6 bacterium/mL	No interference
8	Chlamydia trachomatis	CMCC	10^5-10^6 bacterium/mL	No interference
9	Herpes Simplex Virus Ⅰ	CMCC	10^5-10^6 bacterium/mL	No interference
10	Staphylococcus epidermidis	CMCC	10^5-10^6 bacterium/mL	No interference
11	Mycoplasma genitalium	ATCC	10^5-10^6 bacterium/mL	No interference
12	Mycoplasma hominis	ATCC	10^5-10^6 bacterium/mL	No interference
13	Neisseria gonorrhoeae	CMCC	10^5-10^6 bacterium/mL	No interference
14	Group B strep	ATCC	10^5-10^6 bacterium/mL	No interference
15	Human papilloma virus	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 HSV-Ⅱ, 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. All components of this kit may be toxic, avoid entering mouth.
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.

【Manufacturer】

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