Herpes Simplex Virus (HSV) Type 1/2 Detection Kit (Real-time PCR)

Instruction for Use (V1.1)

[REF] UBP-S01650H

[Specification] 50 tests/kit

[Research Use Only]

This kit is used for qualitative detection of Herpes Simplex Virus Type 1 (HSV1) and Herpes Simplex Virus Type 2 (HSV2) to help detection and treat patients with suspected HSV infections [1-2].

Sexually transmitted diseases (STD) are still one of the major threats to global public health security. Such diseases can lead to infertility, premature fetal delivery, tumor and various serious complications [3-6]. There are many types of STD pathogens, including bacteria, viruses, chlamydia, mycoplasma and spirochetes, among which Neisseria gonorrhoeae, Mycoplasma genitalium, Chlamydia trachomatis, HSV1, HSV2, Mycoplasma hominis, and Ureaplasma urealyticum are common.

Genital herpes is a common sexually transmitted disease caused by HSV2, which is highly infectious. In recent years, the incidence of genital herpes has increased significantly, and due to an increase in risky sexual behaviors, the detection rate for HSV1 in genital herpes has increased and was reported to be as high as 20%-30%. An initial infection with the genital herpes virus is mostly silent without obvious klinical symptoms except local herpes in the mucosa or skin of a few patients. Since genital herpes is characterized by lifelong viral shedding and proneness towards recurrence, it is important to screen the pathogens as soon as possible and block its transmission.

This kit is used to detect HSV1 and HSV2 DNA. The test results shall not be used as the sole outcome measure for evaluation of the patient's condition but must be interpreted in conjunction with the patient's klinical manifestations and other laboratory findings for comprehensive analysis.

[Test Principles]

This kit uses PCR combined with Taqman fluorescent probes. It contains specific primers and probes for fluorescence detection, which are designed to target the conserved regions of HSV1 and HSV2. The HSV1-specific probes are labeled with the fluorophore FAM at the 5'-end, and the quencher BHQ1 at the 3'-end, respectively. The HSV2-specific probes are labeled with the fluorophore CY5 at the 5'-end, and the quencher BHQ3 at the 3'-end, respectively. During PCR amplification, the specific primers and probes separately bind to their own target sequences. When the Taq DNA polymerase encounters the probes binding to their target sequences, it exhibits the exonuclease activity at the 5'-end to isolate the fluorophores (reporter dyes) from the quencher, thereby allowing the fluorescence-monitoring system to receive fluorescence signals, i.e., when a DNA strand is amplified, a fluorescent molecule will be formed to achieve complete synchronization of PCR product formation and fluorescence signal accumulation, thereby qualitatively detecting HSV1 and HSV2 in a sample. The kit contains internal references to control the quality of reagents, DNA and the operation itself to avoid false negative test results.

[Kit Contents]

No.	Name	Specification	Description
1	HSV Reaction Solution	750 μL /vial×1	HSV1/2 specific primers, fluorescent probes, internal control gene primers, fluorescent probe, RT-PCR mix, hot-start DNA polymerase
2	HSV Positive Control	250 μL/vial×1	HSV1/HSV2 and internal control template
3	HSV Blank Control	250 μL/vial×1	RNase-and DNase-free H2O

Noted: The contents in different batches of reagents are not interchangeable within the shelf life.

Reagents required but not provided:

Bacterial Genomic DNA Extraction Kit from ULTRASSAY.

Self-prepared test materials:

RNase and DNase free Tip, disposable gloves, 1.5mL RNase and DNase free centrifuge tube, 0.2mL fluorescent quantitative PCR8 tubes.

[Storage Conditions and Shelf Life]

This kit should be stored below -18℃, and its shelf life is 12 months. After opening and using the kit, store the remaining reagents below -18℃ protected from light. It shall not be subjected to repeated freezing and thawing for more than 4 cycles. After opening it, use it up within 3 months. It can be stored stably for 5 days when it is transported below -18°C under dark conditions.

[Applicable Equipment]

Applied Biosystems 7500 Real-Time PCR Systems, BioRad CFX96 Real- Time PCR System, Ultrassay Archimed X4/X6 Real Time qPCR System

[Acceptable Specimens]

1. Sample collection

Male: Take a urethral secretion, or insert a small cotton swab about 2-4cm into the urethra, then slightly twist it to take out a secretion (which should slightly contain a mucosa). Put the secretion or cotton swab into a sterile tube, then seal it tightly with a sterile cotton ball or rubber stopper, and send it for testing.

Female: Cervix — Use a cotton ball moistened with sterile normal saline to wipe off secretions outside the cervix, then insert a sterile cotton swab into the cervix, and let it stop there for 5 seconds, then slightly twist it to take a cervical secretion (which should slightly contain a mucosa). Put the cotton swab into a sterile tube, then seal it tightly with a sterile cotton ball or rubber stopper, and send it for testing. Urethra — Use a cotton ball moistened with sterile normal saline to clean the urethral opening, then insert a sterile cotton swab about 2cm into the urethra, and slightly twist it to take out a secretion (which should slightly contain a mucosa). Put the cotton swab into a sterile tube, then seal it tightly with a sterile cotton ball or rubber stopper, and send it for testing.

2. Sample storage

Samples to be tested should be stored at 2-8°C for no more than 7 days, at -18°C for no more than 7 months, or at -70°C for a longer time.

Samples should not be subjected to repeated thawing and freezing.

3. Sample transportation

Samples are transported for no more than 5 days using airtight foam boxes with dry ice.

[Test Procedures]

1. Sample processing:

Add 1 mL of sterile normal saline to the sample tube, and shake it well; perform DNA extraction according to the Instructions of Use of the recommended extraction kit. Elute with DNase- and RNase-free water (the recommended elution volume is 100 μL). An extracted DNA sample should be used immediately for testing or stored below -18°C for no more than 7 months, and shall not be subjected to repeated freezing and thawing for more than 4 cycles.

2. Preparation of reagents for amplification

Take out the HSV nucleic acid reaction solution from the kit, and shake to mix it well. According to the number (N) of samples to be prepared (N = number of samples + 1 tube of the positive control + 1 tube of the blank control), add the HSV nucleic acid reaction solution into the 8-tube strip for fluorescence-based qPCR, press the tube cap tightly, and quickly transfer it to the sample processing area.

3. Add sample

Add 10 μL of the DNA sample extracted in Step 1, 10 μL of the blank control and 10 μL of the positive control into the 8-

tube strip, then press the tube cap tightly, and centrifuge at 2,000 rpm for 10 sec. Put the 8-tube strip into the fluorescence-based PCR instrument, and record the loading sequence.

4. The PCR amplification

4.1 Instrument channel and reaction volume selection:

Virus Type	Fluorophore	Quencher
HSV1	FAM	None
HSV2	CY5	None
Internal Control	VIC/HEX	None

Sample Volume is 25. For specific detection channel Settings, refer to the instructions of each instrument.

4.2 PCR amplification conditions:

Step	No. of Cycle	Temperature	Duration	Collect Fluorescent Signal
1	1	50°C	5 minutes	No
2	1	95°C	10 minutes	No
3	40	95°C	15 seconds	No
4		58°C	30 seconds	Yes

5. Result analysis

1. Baseline and threshold setting for ABI 7500 Fluorescence-based qPCR Instrument

Baseline setting: baseline start = 3, baseline end = 15.

Threshold setting: The threshold for each channel should be set separately. When setting the threshold line for a certain channel, first select the negative control, remove the checked automatic threshold line, change the option " √☐Auto" to " ☐Auto", and then manually adjust the threshold line to just exceed the peak of the normal negative control product's amplification curve (irregular noise line).

2. Quality control

1) Blank control: No typical S-shaped curve, and a Ct value of 0 or no Ct value in the FAM, CY5 and VIC/HEX channels;

2) Positive control: A typical S-shaped curve with a Ct value≤30 in the FAM, CY5 and VIC/HEX channels;

3) The above requirements must be met at the same time in the same test, otherwise, such test is invalid. Replicate assays for a sample must be performed strictly according to the procedure in the Instruction for Use.

[Reference Range]

It is determined that the positive judgment value for this kit is a Ct value≤38, and 38 < Ct < 40 is a detection gray zone. The positive judgment value is determined based on the ROC curve with CT values of the klinical sample.

The positive judgment value in the internal reference is a Ct value≤38.

[Explanation of Test Results]

S/N	Ct Value		Interpretation of results
	Target	Internal Control	
1	Ct ≤ 38 for HSV1 (FAM) or HSV2 (CY5)	/	Positive for HSV1 or HSV2
2	No Ct value for HSV1 (FAM) or HSV2 (CY5)	Ct≤35	Negative or below the LOD for HSV1 / HSV2
3	38<Ct<40 for HSV1 (FAM) or HSV2 (CY5)	Ct≤35	A re-test is required: If the re-test shows 38 < Ct < 40, the result is determined as positive. If the re-test shows a Ct value of 0 or no Ct value, then the viral DNA is below the LOD, and it is recommended to take a new sample for testing or verify the result using another procedure.
4	Ct>38 for HSV1 (FAM) or HSV2 (CY5)	Ct >35 or no Ct value	If a test is considered invalid, it is required to re-extract a DNA sample for re-testing and then interpret the results according to above 1-3, and if the results are still Ct > 38 for the target and Ct > 35 or no Ct value for the internal control, then it is determined that the sample fails to meet the requirements.

[Limitations of Test Method]

1. The test results obtained by the kit are for research use only.

2. Any of unreasonable processes of sample collection, transportation and handling/processing may result in inaccurate test results.

3. A negative test does not mean that the patient is not subjected to HSV infection. A final conclusion must be made in conjunction with other results. A negative test may be caused by: ①unreasonable processes of sample collection, transportation and handling/processing, or low pathogen titers in samples; ②variations in target sequences of pathogens; ③other interference factors that have not been verified, such as administration of antibacterial or antiviral; ④infections caused by other viruses or bacteria.

[Product Performance]

1. Positive percent agreement (PPA): The result from the inhouse positive reference standard tested by the kit should be positive;

2. Negative percent agreement (NPA)

2.1 The from the inhouse negative reference standard tested by the kit should be negative;

2.2 The kit should not cross-react with other STD pathogens such as Treponema pallidum, Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum;

3. Repeatability: CV≤5.0% (n=10) for each target in each channel of the repeatability reference standard;

4. Limit of detection (LOD): The LLOD by this kit is no more than 50 copies/reaction.

[Precautions]

1. Samples should be collected, stored, transported and handled according to corresponding national management procedures and biological safety regulations. When samples are handled, relevant protective measures must be taken to ensure the safety of laboratory personnel.

2. When carrying out a test, perform different operations strictly according to different areas: Area 1: Reagent preparation area — to prepare the reagents needed for amplification; Area 2: Sample processing area — to handle samples to be tested and controls (reference standards); Area 3: Testing area — to perform a PCR amplification assay.

3. Items in each area are for exclusive use and shall not be cross-used to avoid contamination; clean the workbench after testing.

4. Before use, all reagents in the kit should be completely thawed and shaken evenly, and then centrifuged briefly.

5. Extracted DNA stored below −18°C should be thawed on ice before loading, and then centrifuged briefly.

6. The 8-tube strip for fluorescence-based qPCR containing the HSV PCR reaction solution should be capped tightly and then rapidly transferred to sample processing area.

7. When a sample is added, it should be completely dropped into the reaction solution, that is, there should be no residue adhering to the tube wall; tighten the cap as soon as possible.

8. After amplification, take out the 8-tube strip immediately and put it into an airtight autoclaved bag for treetment in a

harmless manner.

9. When aliquoting the HSV nucleic acid reaction solution, avoid air bubbles as far as possible. Before loading, check whether each reaction tube is tightly capped to prevent the leakage of fluorescent substances, which may contaminate the instrument.

10. All pipette tips used during testing should be directly put into a tank containing 84 disinfectant for disposal of waste, and sterilized together with other waste items before discarding them.

11. The workbench and various experimental supplies should be regularly disinfected with 75% alcohol or ultraviolet light.

12. All centrifuge tubes and tips used during testing must be free of RNase and DNase.

13. All centrifuge tubes and tips used during testing must be treated in a harmless manner.

[References]

[1] Lu Daqiao, Xiong Bing, Zhou Changchun, Jin Yili. Distribution and resistance of pathogens causing urogenital sexually transmitted infections[J]. Chinese Journal of Nosocomiology, 2016, 26(02):363-365.

[2] Ma Xiuling. Tree atment of mycoplasma and chlamydia infections in the female genital tract (review)[J]. Chinese Journal of Urban and Rural Industrial Hygiene, 2006(06):67-69.

[3] Liu Chaohui, Xue Fengxia. Consensus on treeatment of herpes simplex virus type 1 infection in the female reproductive tract[J]. Chinese Journal of Practical Gynecology and Obstetrics, 2015, 31(09):791-793.

[4] Zhang Dai, Liu Chaohui. Consensus on the treetment of mycoplasma infection in genital tract[J]. Chinese Journal of Human Sexuality, 2016, 25(03):80-82.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
			Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
	Lot Number		Reference Number
	Number of Tests		Any warnings and/or precaution to take

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