Herpes Simplex Virus Type 1 and 2 Nucleic Acid Typing Detection Kit (Fluorescence PCR)

Instructions for Use

Effective Date: Jan 10, 2022

For professional use only.

For in vitro diagnostic use only.

REF BSJ08S1 / BSJ08M1

INTENDED USE

Herpes Simplex Virus Type 1 and 2 Nucleic Acid Typing Detection Kit (Fluorescence PCR) is used for the qualitative detection and discrimination of herpes simplex virus subtype 1 (HSV-1) and herpes simplex virus subtype 2 (HSV-2) in male urethral swab and female cervical swab from suspected cases. The test is indicated for use as an aid in diagnosis of HSV infection in symptomatic patient and should not be used as the sole basis for clinical diagnosis and treatment or management of patients.

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PRINCIPLE

According to the characteristics of nucleic acid sequence of herpes simplex virus type 1 and herpes simplex virus type 2, two sets of specific primers and fluorescent probes were designed by selecting a conserved region within type and specific region between type respectively. The primers and probes in each group could bind to the corresponding target sequence specifically without cross-reaction. At the same time of PCR amplification reaction, the fluorescent signal generated by the excitation light of the fluorescent probe can be detected by the automatic fluorescent PCR detector to realize real-time online monitoring of the PCR reaction. In the extraction and detection process, human RnaseP gene (IC) is introduced as a non-competitive internal reference to control the whole extraction and detection process. In addition, UDG enzyme anti-contamination measures are added in this kit to fully decompose possible product contamination and avoid false positive results.

COMPONENTS

Components		Main Ingredients	BSJ08S1	BSJ08M1
			24 tests/kit	48 tests/kit
Amplification reagent	PCR Buffer	dNTPs, Mn^{2+}, DNA polymerase and UDG enzyme	312 μL×1	624 μL×1

	HSV Primer / Probe	Specific Primers and Probes	48 μL×1	96 μL×1
Control	Positive Control	A mixture containing HSV-1 plasmid, HSV-2 plasmid and IC plasmid	1000 μL×1	1000 μL×1
	Negative Control	IC plasmid	1000 μL×1	1000 μL×1

a. *The positive control and negative control need to be set to monitor the test body and the operating environment; the negative control and positive control have been packaged in the kit.*
b. *The components of different lots cannot be mixed for use.*
c. *Equipment or materials required but not provided: Specimen collection kits, Nucleic acid extraction kits; PCR tubes and caps, etc.*

APPLIED INSTRUMENT

The kit can be applied to Fluorescent Quantitative Detection System from Hangzhou Bioer Technology Co., Ltd, QuantGene 9600 (FQD-96C) and LineGene 9600 Plus (FQD-96A). The instrument should contain at least three channels of FAM, HEX and CY5.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use (IVD).
- Read the Instructions for Use carefully before operation. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC). Handling samples in the biosafety cabinet, to ensure operator safety and avoid environmental pollution. Place harmful samples and reagents properly. Discard the waste in special containers. Wipe the table, centrifuge, and equipment frequently with 1.0% sodium hypochlorite or 70 % ethanol. The laboratory and the ultra-clean workbench need UV-treated periodically and after each experiment.
- All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
- Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents, while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.

- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product.
- Separate laboratory areas are recommended to performing predefined procedures of the assay. Area I: Reagent preparation area-reagent required for preparing amplification. Area II: Sample processing area-processing of tested samples and controls. Area III: PCR detection region-PCR amplification detection.
- The separation of the reaction solution should avoid the generation of air bubbles as far as possible. Before the amplification, pay attention to check whether the caps of each reaction tube are tightened to avoid contaminating instrument.
- Samples should be completely put into the reaction solution when adding samples. No samples should adhere to the tube wall and the cap should be tightened as soon as possible after adding samples.
- Both the kit and nucleic acid products are all stored at -20°C. Before using, they should be fully thaw out at room temperature, mixed and then instantaneous briefly centrifugation.
- After amplification, please take out the reaction tube immediately, seal it in the special plastic bag, put it in the designated place, and wait for unified treatment.
- Dispose of used / unused kit reagents and human specimens according to local, state, and federal regulations.

STORAGE AND PERIOD OF VALIDITY

1. The kit should be stored at -25°C ~ -15°C away from light and avoid repeated freeze-thaw. The kit can be stored for 7 days at 2 ~8 °C after opening.
2. The kit can be stored for up to 12 months if all components are kept in the manner above. Do not use after the stated expiry date.
3. The kit can be transported in foam box sealed with ice bags or dry ice at 2 ~8°C or lower.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION

1. Specimens: Human cutaneous and mucocutaneous lesion swabs, such as urethral swab, cervical swab, swab of herpes ulcer, etc.
2. Collection: Specimens of all types are collected by conventional methods.
3. Storage: It is recommended that specimens be processed as soon as possible after collection. If specimens are not processed immediately, they should be stored at 2~8 °C for up to 72 hours. If a delayed processing is expected, the specimens should be stored at -20°C or lower. Specimens should not be frozen and thawed frequently.
4. Transportation: Specimen should be transported with 0°C curling bottle or foam box sealed with ice.

SPECIMEN PRETREATMENT (SPECIMEN DISPOSAL AREA)

Follow the instructions of the nucleic acid extraction and purification kit.

For Automatic extraction: It is recommended to use MagaBio plus Virus DNA/RNA Purification Kit III (Cat: BSC86) or MagaBio plus Virus DNA/RNA Purification Kit II (Cat: BSC71) to purify the nucleic acid with Gene Pure Series Nucleic acid extractor.

⚠ **Note: The negative control, positive control and unknown specimen need to be tested in the same experiment.**
It's recommended to prepare the reagent ahead of specimen pretreatment to ensure that the reagents are not contaminated.

USING OF THE KIT PCR REACTION (PCR TEST AREA)

1) Reagent prepares

Thaw out the reagents at room temperature. Mix gently and centrifuge all reagents for a few seconds.

Prepare reagents according to the quantity of specimens and controls as below (N means the number of **specimens and controls**):

Make sure that at least one positive control and at least one negative control is used per run.

Reagents	Dosage/ test	Dosage
PCR Buffer	13 μL	N×13 μL
HSV Primer/Probe	2 μL	N ×2 μL

Distribute15 μL mixed reagents (13 μL PCR Buffer and 2 μL HSV Primer/Probe) into each PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

Add 10 μL negative control, 10 μL extracted product and 10 μL positive control into different PCR tube. Cap the PCR tubes immediately to prevent cross contamination.

⚠ **Note: Do not label on the scanned area of the reaction tubes!**

3) qPCR reaction

Place the reaction tubes on a PCR instrument.

It is recommended to choose FAM, HEX and CY5 channels to collect fluorescent signals.

Set fluorescent signals detecting at 60 °C, liquid volume is 25 μL.

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	37°C	2 min	1
2	95°C	1 min	1
3	95°C	5 sec	45
	60°C	10 sec	

QUALITY CONTROL STANDARDS

Expected performances of controls are as below:

Control	FAM	HEX	CY5	Interpretation of Test Results
Positive Control	All the three channels yield Ct Value ⩽35 with "S" amplification curve			All requirements are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct Value		Ct Value⩽35 with "S" amplification curve	

RESULT ANALYSIS AND JUDGMENTS

Expected performances of specimens are as below:

FAM (HSV-2)	HEX (HSV-1)	CY5 (IC)	Result Judgment
Ct Value ≤39.8, with "S" curve	Ct Value ≤39.2, with "S" curve	No specific requirement	Both HSV-1 and HSV-2 nucleic acid Positive.
Ct Value ≤39.8, with "S" curve	Ct Value >39.2, or no Ct Value		HSV-2 nucleic acid Positive.
Ct Value >39.8, or no Ct Value	Ct Value ≤39.2, with "S" curve		HSV-1 nucleic acid Positive.
Ct Value >39.8, or no Ct Value	Ct Value >39.2, or no Ct Value	Ct Value ≤40, with "S" curve	Both HSV-1 and HSV-2 nucleic acid Negative.
Ct Value >39.8, or no Ct Value	Ct Value >39.2, or no Ct Value		Invalid, resample.

Note:

a) A valid test is a test where the Internal Control (CY5 Channel) result is positive, or the Internal Control (CY5 Channel) is negative, but at least one of the two target channels (FAM and / or HEX) is positive.

b) Results should always be used in combination with other medical finding, such as

symptoms, results of other tests, clinical impressions, etc. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

c) Inconclusive or the invalid result could be caused by 1) handling error (e.g., sample collection, DNA extraction) or inhibition; 2) a sample at concentrations near or below the limit of detection of the specific target; 3) a mutation in the corresponding target region; 4) infection with some other unknown virus; 5) other factors.

d) For the inconclusive result or the invalid result, retesting must be performed by re-extraction the original sample and repeating the PCR. If the repeated result remains invalid, it's recommended to report the result to the sender and collect a new specimen and /or perform an additional confirmatory testing.

LIMITATIONS

1.This kit is only used for qualitative in vitro auxiliary detection the presence of HSV-1 and HSV-2. Neither the quantitative value nor the rate of increase can be determined by the qualitative test.

2. The results of the test are just for clinical reference. The test should not be used as sole criteria for diagnosis. Results should be considered in conjunction with the clinical information and other data available to the physician.

3. The quality of specimen obtained is of extreme importance. Good laboratory practices and changing gloves between handling patient specimens are recommended to avoid contamination of specimens or reagents. An incorrect result may occur by incorrect operation in sample collection, transportation, or processing.

4. Possibility analysis of false negative results:(1) unreasonable sample collection, processing, transportation, storage conditions and improper experimental operation; (2) The nucleic acid concentration of the target substance in the sample is lower than the detection limit of the kit;(3) Detect the mutation of the target gene sequence of the virus or the sequence change caused by other reasons;(4) Unverified other interfering substances, such as endogenous or exogenous substances introduced into the sample.

5. Possibility analysis of false positive results: (1) cross-contamination between samples; (2) Other unverified cross-reactive substances.

6. For the positive result or any suspected cases, it's recommended to re-extract and / or retest with a new lot of kit or confirmed with another available method.

7. Due to the limitation of detection threshold and detection range, negative results do not preclude infection with herpes simplex virus. The test result should not be the sole basis of a patient management. Follow up testing/ analysis should be performed.

PERFORMANCE INDICATORS

Performance validation was conducted with Line-Gene 9600 and Quantgene 9600 series fluorescent quantitative PCR detection system from Bioer. The kit can be

applied to Bioer's Line-Gene 9600 and Quantgene 9600 series fluorescent quantitative PCR detection system and other manufacturers' similar fluorescent quantitative PCR detection systems.

The positive references used for validation were virus cultures of herpes simplex virus type 1 and herpes simplex virus type 2, which were traced back to ATCC.

- ★ Limit of Detection (LoD): Cultures of herpes simplex virus type 1 and herpes simplex virus type 2 were diluted to 1000 copies/mL,500 copies/mL, 200 copies/mL and 100 copies/mL with negative samples, then were tested by 3 lots of kits. Each concentration was tested with 20 replicates. The testing data demonstrated that the kit can detect herpes simplex virus type 1 and herpes simplex virus type 2 with detection more than 95% at the concentration equal or higher than 200 copies/mL.
- ★ Analytical sensitivity: The positive reference standards and negative reference standards were tested by 3 lots of kits. The positive coincidence rate was 100%, and the negative coincidence rate was 100 %.
- ★ Analytical specificity: No cross reactivity has been observed by testing the clinical positive specimens such as Group B streptococcus, HPV16 , HPV18,Salmonella,Ureaplasma urealyticum, Chlamydia trachomatis, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Escherichia coli, Varicella - zoster virus, Human cytomegalo virus, Epstein-Barr virus, Human herpes virus type 6, Human herpes virus type 7, Pneumonia mycoplasma, Pertussis coli, Staphylococcus aureus, Candida albicans, Candida glabrata ,B hemolytic streptococcus and Adenovirus.
- ★ Analytical specificity: The potentially interfering substances were spiked into positive control, then tests were performed with a lot of kits. The tested substances Blood (10%), mucin (0.2mg/mL), cervical mucus (15%) respectively, vaginal lubricant (3%), fuyan cleansing solution (5%), azithromycin (0.4mg/L), levofloxacin (5μg/mL) showed no influence on the detection.
- ★ Precision: Positive controls and low positive controls were tested by 3 lots of kits with 10 replicates by 2 operators for 20 days. The results showed that the variation coefficient (CV) of within-lot, between-lots, within-days and between-days were less than 5%.

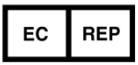
REFERENCES

[1] Ridker P M, Hennekens C H, Stampfer M J , et al. Prospective Study of Herpes Simplex Virus, Cytomegalovirus, and the Risk of Future Myocardial Infarction and Stroke[J]. Circulation, 1998, 98(25):2796.

[2] Fatahzadeh M, Schwartz R A. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management.[J]. Journal of the American Academy of Dermatology, 2007, 57(5):737-763.

[3] Brown Z A, Benedetti J , Ashley R , et al. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor.[J]. N Engl J Med, 1991, 324(18):1247-1252.

SYMBOL DESCRIPTION

	Manufacturer		Catalogue number
	CE mark		Authorized representative in the European community
	Batch code		Consult instructions for use
	In vitro diagnostic medical device		Temperature limitation
	Caution		Use by date
	Positive Control		Negative Control

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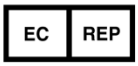

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