Monkeypox Virus Nucleic Acid Detection Kit (Fluorescent PCR)

Instructions for Use

Effective Date: May 23, 2022

For professional use only.

For in vitro diagnostic use only.

IVD -25℃ -15℃ CE

REF BSJ32S1 / BSJ32M1

INTENDED USE

Monkeypox Virus Nucleic Acid Detection Kit (Fluorescent PCR) is used for the qualitative detection of the monkeypox virus nucleic acid in specimens of lesion surface swabs, lesion exudate or whole blood from suspected cases.

Positive results are indicative of the presence of monkeypox virus nucleic acid, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out co-infection with bacterial or other viruses. Negative results do not preclude monkeypox virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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PRINCIPLE

This product selects the Monkeypox Virus (FAM) designs one sets of primers and fluorescent probes. The one set of primers and probes can specifically bind to the target sequences. When the PCR amplification reaction is performed, the fluorescent signal(s) can be detected by a full-automatic fluorescent PCR detector to realize real-time online monitoring of the PCR reaction. In order to control the entire extraction and detection process, human gene was act as a non-competitive internal control during the extraction and detection process.

COMPONENTS

Components		Main Ingredients	BSJ32S1	BSJ32M1
			24tests/kit	48tests/kit
Amplification reagent	MPXV PCR Buffer	dNTP, Mg2+, DNA polymerase	312μL×1	624μL×1
	MPXV Primer/Probe Mix	Specific Primers and Probes of MPXV	48μL×1	96μL×1

Control	MPXV Positive Control	Plasmid with specific genes and internal reference gene	1mL×1	1mL×1
	MPXV Negative Control	Plasmid with internal reference gene	1mL×1	1mL×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 Hangzhou Bioer Technology Co., Ltd. QuantGene 9600 Fluorescent Quantitative Detection System (FQD-96C) and LineGene 9600 Plus Fluorescent Quantitative Detection System (FQD-96A). The instrument should contain at least two channels of FAM and CY5.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use (IVD). For professional use only.
- 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. RNA should be maintained on cold-block or on ice during preparation and use to ensure stability.
- 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 3 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: specimens of lesion surface swabs, lesion exudate or whole blood.
2. Collection: Specimens should be 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 24 hours. If a delayed processing is expected, the specimens should be stored at -70°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 II (Cat: BSC71) or MagaBio plus Virus DNA/RNA Purification Kit III (Cat: BSC86) 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.

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

Reagents	Dosage/ test	Dosage
MPXV PCR Buffer	13μL	(N+1) ×13μL
MPXV Primer/Probe Mix	2μL	(N+1) ×2μL

Distribute 15 μL mixed PCR reagents into each PCR tubes, and then transfer the reaction plate to sample processing area.

2) Adding sample

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

contamination.

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

3) PCR reaction

Place the reaction tubes on a PCR instrument.

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

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

Set reaction procedure as below:

Step	Temperature	Duration	Number of cycles
1	37°C	1 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	CY5	Interpretation of Test Results
Positive Control	FAM and CY5 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 (MPXV)	CY5 (Internal Control)	Result Judgment
Ct Value ≤40 with "S" curve	No specific requirement	Monkeypox Virus nucleic acid Positive.
Ct Value ＞40, or no Ct Value	Ct Value ≤40, with "S" curve	Monkeypox Virus nucleic acid Negative.
Ct Value ＞40, or no Ct Value	Ct Value＞40，or no Ct Value	Invalid, re-sample.

NOTE:

1. When the specimen test result is invalid, it needs to be re-sample and tested.

LIMITATIONS

1. The kit is only used for the qualitative detection the presence of monkeypox virus in specimens. 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. Negative result does not preclude monkeypox virus infection and should not be used as the sole basis for the diagnosis, treatment or other patient management decisions.
3. An incorrect result may occur by incorrect operation in sample collection, transportation or processing.
4. A false negative result may occur by very low concentration of target virus in the specimens, mutations within the viral genome covered by the kit's primers and/or probe, and unproved external interference factors, such as PCR inhibitor.
5. A false positive result may occur by aerosol pollution or operating errors.
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.

PERFORMANCE INDICATORS

Performance validation was conducted with Hangzhou Bioer Technology Co., Ltd. QuantGene 9600 Fluorescent Quantitative Detection System (FQD-96C) and LineGene 9600 Plus Fluorescent Quantitative Detection System (FQD-96A). Since clinical positive specimen was fewer, positive reference standards was prepared for the validation. The positive control was monkeypox virus plasmid, which was purchased from a commercial company.

★ Limit of Detection (LoD): The positive reference standard was diluted into 2000 copies/mL, 1000 copies/mL, 500 copies/mL, 200 copies/mL and 100 copies/mL, then were tested by 3 lots of kits. Each concentration was tested with 20 replicates. The testing data demonstrated that the kit can detect monkeypox virus with detection rate equal or higher than 95% at the concentration equal or higher than 500 copies/mL.

★ Analytical sensitivity: 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 Enterovirus 71, Measles Virus, Rubella Virus, Varicella Zoster Virus, Herpes Simplex Virus I, Herpes Simplex Virus II, Epstein-Barr Virus, Cytomegalovirus, Human Herpesvirus Type 6, Human Herpesvirus Type 7 and Human genomic DNA etc.

★ Analytical specificity: The potentially interfering substances were spiked into positive control, then tests were performed by 1 lot of kits. The tested substances Blood (10%), Mucins (0.2mg/mL) , Oxymetazoline (0.5mg/L), Sodium chloride (0.09%), Dexamethasone (0.1mg/L), Triamcinolone acetonide (105ng/mL), Budesonide (3nmol/L), Mometasone (0.03%), Fluticasone (0.5ng/mL), Ribavirin (3680ng/mL), Oseltamivir (1275μg/L), Levofloxacin (5μg/mL), Azithromycin (0.4mg/L), Tobramycin (3.7μg/mL), Phenylephrine (0.5mg/mL), Beclomethasone (0.2mg/L), Flunisolide (1mg/mL), Histamine hydrochloride (1mg/mL), Zanamivir (142ng/mL), Peramivir (100μg/mL), Lopinavir (25μg/mL), Ritonavir (25μg/mL), Arbidol (614.1ng/mL), Ceftriaxone (80μg/mL), Meropenem (100μg/mL) showed no influence on the detection.

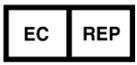
★ Precision: Positive controls and low positive controls were tested by 3 lots of kits with 8 replicates by 2 operators for 5 days. The results showed that the variation coefficient (CV) of within-lot, between-lots, between-operators and between-days were less than 5%.

REFERENCES

[1] About Monkeypox, CDC, www.cdc.gov/poxvirus/monkeypox/about.html.

[2] Multi-country monkeypox outbreak in non-endemic countries, WHO, May 21, 2022, www.who.int/emergencies/disease-outbreak-news/item/2022-DON385.

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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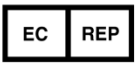

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