Monkeypox Virus Real-time PCR Kit

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures

【PRODUCT NAME】

Monkeypox Virus Real-time PCR Kit

【PACK-SIZE】

50 tests/kit

【INTENDED USE】

This product is intended for the rapid detection of Monkeypox virus in human throat swabs, blister fluid samples.

Monkeypox virus (MPXV) is a brick-shaped, dsDNA virus surrounded by a lipoprotein envelope. On electron microscopy, the MPXV is relatively large (200-250 nanometers). Aside from their reliance on host ribosomes for mRNA translation, poxviruses include all necessary replication, transcription, assembly, and egress proteins in their genome. MPXV is a zoonotic orthopoxvirus that incidentally causes disease in humans similar to smallpox, although with notably lower mortality. This virus is clinically relevant because it is endemic to western and central Africa, with outbreaks in the Western Hemisphere related to the exotic pet trade and international travel. MPXV is from the family: Poxviridae, subfamily: chordopoxvirinae, genus: orthopoxvirus, and species: Monkeypox virus.

MPXV's incubation period is typically about 6–16 days but can vary from 5 to 21 days. There are two facets of the contagious era, with an initial intrusive duration in the first 5 days, where the main signs are fever, lymphadenopathy , back pain, extreme headache, myalgia and serious asthenia. A maculopapular rash occurs 1–3 days after the onset of fever, and grows into small fluid-filled blisters, which are pus-filled and then crust over in about ten days.

【PRINCIPLE OF DETECTION】

This product is a fluorescent probe-based TaqMan real-time PCR assay system. During the amplification of the template, the TaqMan probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The MPXV will be detected qualitatively by FAM channel and the internal control (IC) will be detected by CY5 channel.

dUTP and UNG enzyme are used in the kit to prevent contamination of the amplified products.

Internal control is used in the kit for quality control starting from sample collection.

【PRODUCT CONTENTS】

Components	Amount	Amount per reaction
	50 Tests/kit	
MPXV Reaction Reagent	500 µL	10 µL
MPXV Positive Control	400 µL	-
Negative Control	400 µL	-

Note: Do not mix the components from different batches for detection. The positive control of MPXV and IC were constructed artificially, and they were not infectious.

【STORAGE & SHELF LIFE 】

All reagents should be stored at -15℃~-25℃ with sealed protection from light, and the reagents are stable for 12 months (to be determined) when stored at the recommended condition. See label for production date and expiration date.

The kit should be transported by cold chain transport or sealed foam box with ice. The temperature should be controlled below -8°C and the transportation time should not exceed 4 days at room temperature. Repeated freeze-thaw should be less than 5 times.

【INSTRUMENTS】

Real-time PCR instruments with FAM and Cy5 detection channels—— QuantStudio® 5, 7500 and SLAN96S.

【SAMPLING & HANDING】

Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing physiological saline, discard the tail, and tighten the tube cover.

Blister fluid: Disinfect the skin lesions with alcohol cotton balls and allow to dry naturally. Aspirate the liquid with a sterile needle and syringe, place it in a 400 ul PBS-containing screw-top tube with an O-ring rubber, and tighten the tube cap。

The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.

The sample can be stored for 24 hours at 2~8℃ and for a long time below -20℃.

Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Reagent Preparation

The MPXV reaction reagent was taken out from the refrigerator and thawed, vortexed, and centrifuged briefly. Prepare reagent with ice box, and prepare reaction reagent according to the number of reaction samples (number of reaction samples, n = number of samples to be tested + 2 control samples + 1):

Add n × 10 μL of MPXV Reaction Reagent into different PCR reaction tubes. The reaction tubes can be placed at 2~8℃ for 3 hours after separation.2.

2. DNA Extraction

It is recommended to use the Nucleic Acid extraction and purification reagent (general type) produced by our company to extract DNA from sample, Positive and Negative control.

The volume of sample to be extracted is 400 μL; after DNA extraction, the extracted DNA shall be added to the reaction tubes within 10 minutes, or transferred to the centrifuge tubes and stored at -15 ℃~-25 ℃.

3. Template Addition

Add 10 μL of extracted Negative Control, 10 μL of extracted Positive Control, and 10 μL of extracted DNA from sample to different PCR reaction tubes. Centrifuge them at low speed. Then, move them to the Real-time PCR instrument.。

4. PCR Amplification

QuantStudio® 5

Step1: 50℃ for 2minutes, 1 cycle；

Step2: 95℃ for 30 seconds, 1 cycle；

Step3: 93℃ for 1 seconds to 60℃ for 10 seconds, 40 cycles. The signals of FAM and CY5 fluorescence channels will be collected at 60℃.

7500 and SLAN96S

Step1: 50℃ for 2minutes, 1 cycle；

Step2: 95℃ for 30 seconds, 1 cycle；

Step3: 93℃ for 1 seconds to 60℃ for 31 seconds, 40 cycles. The signals of FAM and CY5 fluorescence channels will be collected at 60℃.

5. Data Analysis

Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM and CY5 channels respectively.

(1) Baseline setting: the baseline can be set automatically or adjusted according to the shape of amplification curve.

(2) Threshold setting: the threshold value should be higher than the highest fluorescence value of negative control in this kit.

6. Quality Control

Negative control and positive control provide the calibration for the kit, and shall be set for each test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

Products of Quality Control	Requirements of Quality Control	
	FAM Channel	CY5 Channel
Positive Control of MPXV	Ct ≤ 35	No requirement
Negative Control	Undet or Ct>39	No requirement

7. Interpreting Test Results

According to channel detection results, the judgment results are as follows:

Test Results	Interpreting Test Results
FAM has amplification signal, Ct ≤ 39, and amplification curve is typical S shape, then FAM Channel (+)	MPXV (+)
FAM has no signal, or Ct >39, and CY5 has amplification signal, Ct ≤ 39, then FAM Channel (-)	MPXV (-)
The Ct of FAM is more than 39 or no value; and the Ct of CY5 is more than 39 or no value	There is a problem with the sample or operation, which needs to be retested.

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off value of MPXV is Ct ≤ 39.

【ASSAY EXPLAINATION】

1. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.

2. The decrease of detection effect even the false negative result may occur if there is any mistake in the transportation, storage and operation of reagents.

3. MPXV early infection or other orthopoxvirus infection can't be excluded in patients with negative results. If conditions permit, it is recommended to collect different samples for retest.

【ASSAY LIMITATIONS】

1. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.

2. This kit is intended for detection of MPXV.

3. Although the detected target sequences of this kit are the conservative region of MPXV's gene, the missed detection of orthopoxvirus with rare mutations in the conservative region can't be completely avoided in theory.

【PERFORMANCE SPECIFICATIONS】

1. Detection limitation: 125 copies/mL.

2. Specificity: non-specific interference of other related orthopoxvirus pathogens.

【ATTENTIONS】

1. The kit is for Research Use Only. Not for use in diagnostic procedures.

2. Please read this manual carefully before beginning the experiment.

3. All equipment used in the experiment shall be sterilized.

4. Unreasonable sample collection, transfer, storage and operation may lead to wrong test results.

5. DNA extraction shall be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it shall be stored in accordance with [SAMPLING & HANDING].

6. After the operation of the nucleic acid extractor, the used consumables shall be sealed. After the instrument is cleaned, turn on the ultraviolet lamp for 30 minutes.

7. As this test involves the extraction of viral DNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Regular monitoring of laboratory contamination is recommended.

8. When using this kit, please strictly follow the instructions. The collection, storage and transfer of samples, the extraction and detection of DNA, and the interpretation of results must be carried out in strict accordance with the requirements of the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the clinical gene amplification laboratory.

9. MPXV has strong transmission ability and high-risk coefficient. Personal protection should be a three-level laboratory level of biosafety. The operator must have professional skills and PCR inspection qualification. During the whole operation process, it is necessary to prevent the infection risk of aerosol pollution, and the operator must add samples and use reagents and consumables accurately.

10. To prevent virus spreading, the MPXV must be detected in a biosafety level 2 (P2) or above laboratory.

Laboratory management should strictly follow the management standard of PCR gene amplification laboratory, and the experimental operation must be strictly partitioned. The instruments, equipment, consumables, work clothes used in each region must be distinguished strictly and can't be used intercross to avoid contamination.

11. All test samples shall be regarded as infectious substances. During the experiment, work clothes shall be worn, disposable gloves shall be worn and replaced frequently to avoid cross contamination between samples. The operation of sample and waste shall meet the requirements of relevant laws and regulations such as The general guidelines for biosafety of microbiological biomedical laboratories and The regulations on the management of medical wastes issued by the Ministry of Health.

【Reference】

[1] WHO. Laboratory testing for the Monkeypox virus (interim guidance). 2022.
[2] McCollum AM, Damon IK. Human Monkeypox. Clinical Infectious Diseases. 2014 Jan 15;58(2):260–7.
[3] Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. Journal of Virological Methods. 2010 Oct;169(1):223–7.

【General Information】

Manufacturer: Fosun Diagnostics (Shanghai) Co., Ltd.
Address: No.830 Chengyin Road, Baoshan District, 200444 Shanghai, PEOPLE'S REPUBLIC OF CHINA.
Contact information：+86-21-60765888 +86-21-60765999