Monkeypox Virus (MPXV) RT-PCR Kit

Catalog No.: abx471001

Size: 300 tests

Limit of Detection: 10 copies/μL

Storage: Store at -30 °C to -15 °C.

Application: For the qualitative detection of monkeypox virus in lesion exudate specimens.

Introduction

This kit uses the conserved sequence of the F3L gene from the monkeypox virus as the target region for multiplex real-time PCR amplification. In order to achieve the qualitative detection of the monkeypox virus at the nucleic acid level, the fluorescent quantitative PCR device has the ability to automatically generate a real-time amplification curve based on the detected fluorescent signal. Two primers and probes are included in the multiplex real-time PCR detection system for internal control (RNase P). For each test run, the kit's positive and negative controls can be utilized as an external control. It is possible to monitor proper specimen collection, handling, and real-time PCR process using the outcomes of the internal and external control.

Kit components

1. PCR reaction buffer: 1mL
2. Positive Control: 0.5 mL
3. Negative Control: 0.5 mL

Materials Required But Not Provided

1. Extraction Buffer: 1 mL (**optional**)
2. Biosafety cabinet
3. General laboratory equipment
4. Real time PCR system
5. Nucleic acid extraction kit and instrument Pulse centrifuge
6. Vortex mixer
7. Real time PCR reaction tubes (0.2 mL)
8. Ice-container
9. Transfer pipettes (0.5 μL-1000 μL)
10. Sterile tips for transfer pipettes
11. Sterile tubes
12. Biohazard waste container
13. Refrigerator and freezer

Protocol

A. Sample Collection and Preparation

1. Collecting Specimen

Using a swab, wipe the lesion, rotate, and remain there for ten to thirty seconds to enable the swab to absorb all secretions. The specimen must be gathered into sterile, labeled tubes and sent in accordance with the specifications of the testing lab.

2. Specimen Storage

Testing should be done as soon as the specimen is collected. The samples to be tested should be stored at 2–8°C for no more than 24 hours if they are not tested right away. They can be kept for a long time below -70°C, and at -20°C for no more than three months.

It is important to prevent damaging specimens through proper storage, as frequent thawing and refreezing can lead to erroneous results.

Note:

Each work area; the areas for preparing specimens, storing and preparing chemicals, and amplifying data should be kept apart to avoid cross-contaminating specimens.

B. Assay Procedure

1. **Reagent Storage and Preparation Area**
   - 1.1 Thaw the kit components at 4°C to 30°C.
   - 1.2 Please make sure the extraction buffer dissolves completely and returns to room temperature before using the kit (in the event of precipitation, incubate at 56°C for 5 minutes to dissolve completely).
   - 1.3 Thaw the PCR reaction buffer at room temperature, fully thaw, shake and mix well, centrifuge atlow speed for a few seconds, and then divide the solution into PCR reaction tubes according to the number of samples to be amplified (sufficient for the number of patient samples and controls). Cap the PCR reaction tubes with care, ensure that the caps are placed correctly, and transfer to the specimen preparation area, store in a refrigerator at 4°C away from light.

Note: It is important to vortex the reagents. Compensational pipetting is recommended.

2. **Specimens Preparation Area**
   - 2.1 DNA is extracted according to the instructions for use of the extraction kit
   - 2.2 Add 1 mL of normal saline to the sample collection tube, shake the cotton swab sufficiently to wash it, then squeeze the cotton swab against the tube wall and discard , transfer 500 µl of liquid to a 1.5 mL tub, then centrifuge for 5 minutes at 12,000 rpm. Discard the supernatant and collect the sediment.
   - 2.3 Add 1 mL of normal saline to the sediment, shake on a vortex shaker to disperse the sediment. Centrifuge the contents at 12,000 rpm for 5 minutes and discard the supernatant, and collect the sediment.
   - 2.4 Using the prepared extraction buffer, add 50 µl and shake on a vortex shaker to disperse the sediment. Dry or place the contents in a water bath at 100 °C for 10 minutes. Then centrifuge at 12,000 rpm for 5 minutes, and remove the supernatant solution for PCR reactions.

2.5 The isolated DNA must be utilized for detection promptly; otherwise, it should be preserved at -20°C. Upon reusing the extracted DNA, it is essential to completely thaw it and centrifuge at 12,000 rpm for 5 minutes, after which the supernatant can be employed for the PCR reaction.

2.6 If the extracted DNA is not used immediately, it can be stored at 2-8 °C for no more than 24 hours

3. Addition of Samples

3.1. Pipette 5 μL of each purified DNA sample , negative and positive controls into the designated PCR reaction tube containing the PCR reaction buffer.

3.2. Cap the PCR reaction tubes with care, ensuring that the caps are placed correctly.

3.3. Vortex the PCR reaction tubes briefly and centrifuge to collect the solutions at the bottom.

4. **Amplification Area**

4.1 Place the reaction tubes with a volume of 25 μL into the PCR instrument, configure the settings, and initiate the specified cycling protocol.

	Process	Cycles	Temperature (ºC)	Time (s)
Step 1	Initial Denaturation	1	95	300
Step 2	Denaturation	40	95	10
Step 3	Annealing, extension and testing		60	30

4.2 Settings of detection fluorescence

Channel	Target Gene
FAM	F3L gene of Monkeypox virus
VIC	Rnase P

4.3 adjust the internal reference parameter for fluorescence on the instrument to "None." For instance, in the case of the ABI 7500, please configure the "Passive Reference" setting to "None."

4.4 Initiate the PCR cycler in accordance with the instructions provided in its user manual.

C. Result Analysis

For the ABI 7500, establish the baseline within the range of 3-15, allowing for adjustments based on the specific circumstances. The principle for setting the fluorescence threshold is to position the threshold line just above the peak value of the negative control amplification curve, which is represented by the irregular noise line. In this case, the Ct value will be indicated as Under. Utilize the instrument's software for automatic result analysis.

- Negative control: no obvious S-shaped amplification curve, and Ct value is shown as Under.
- Positive control: both channels have typical S-shaped positive amplification curves, and the Ct value is less than or equal to 30.

Note: The above two conditions must be satisfied at the same time, otherwise, this test is invalid.

Reference Value

1. Ct value is displayed as under, negative result.
2. Ct value < 38, positive result.

Interpretation of test results

Assuming the experiment is valid, the test results are evaluated based on the table provided below.

Table.1 Ct value of each fluorescence channel and judgment of negative and positive results

Fluorescence channel (target gene)	Ct Value	Interpretation
VIC (internal reference)	CT ≤ 40	Negative
	Ct < 40, With an identifiable S-type amplification curve	Positive
FAM Monkeypox Virus	CT≤40	Negative
	Ct < 38 With an identifiable S-type amplification curve	Positive
	38 ≤ Ct < 40 With an identifiable S-type amplification curve	In order to identify the grey area, it is necessary to conduct a repeat measurement. If the Ct value obtained from the retest is below 40, it is considered positive; conversely, if the Ct value from the retest is 40 or lower, it is deemed a negative result.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.