USER GUIDE

ECALBIO® Monkey Pox Virus qPCR Kit

CAT#: A-PR001

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1 Principle And Intended Use

This test is a qualitative real-time fluorescent PCR that enables to amplification of MPV from the oropharyngeal swab, nasopharyngeal swab, serum, plasma, and human pustular or vesicular rash specimens. Specific primers & probes are designed to detect the highly specific regions of the MPV. A pair of primers and a probe for detecting endogenous human genes are included as an internal control to monitor the whole test process and control for inhibition. The specific probe of the MPV gene is labeled with FAM, and the specific probe of the human gene is labeled with VIC.

The virus DNA in the sample should be purified and enriched with a nucleic acid extraction and purification reagent before being detected with this kit.

The Monkeypox Virus Detection Kit (Fluorescence PCR Method) is a qualitative in vitro real-time PCR test for the detection of nucleic acid from monkeypox virus (MPV) in the oropharyngeal swab, nasopharyngeal swab, serum, plasma, and human pustular or vesicular rash specimens from individuals suspected of MPV infection.

Results are for the identification of MPV DNA. Positive results are indicative of the presence of MPV DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude MPV 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.

The Monkeypox Virus Detection Kit (Fluorescence PCR Method) is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

2 Specifications

96 tests/kit

3 Components

Each kit contains 1 pre-packaged reagent 96-well plate, 2 microplate sealers,1 MPV Positive Control,1 MPV Negative Control, and 1 instruction.

4 Storage Condition and Expiring Date

The reagents are stable when stored at room temperature for 12 months without unpacking. Please transport at -25℃ to 35℃. The kit should remain sealed and away from light exposure. Do not use the kit beyond the expiration date.

5 Applicable Instruments

Bio-Rad CFX96 Touch Real-Time PCR Detection System; Applied Biosystems™ 7500 Real-Time PCR system; Applied Biosystems™ Quant Studio 5 Real-Time PCR system; Applied Biosystems™ QuantStudio 3 Real-Time PCR system; Applied Biosystems™ QuantStudio 7 Flex/Pro Real-Time PCR system; Applied Biosystems™ ViiA7 Real-Time PCR system.

6 Materials Required but Not Provided

① Nucleic acid purification and extraction reagent.
② Pipettes and Pipetting tips (200 μL and 1mL tips with filters)
③ Centrifuge
④ Desktop vortex mixer
⑤ Disposable powder-free gloves and surgical gowns

7 Specimen Requirements

① Sample type

Oropharyngeal swab, nasopharyngeal swab, serum, plasma, and human pustular or vesicular rash specimens.

② Specimen collection

Human pustular or vesicular rash specimens can be collected in tubes containing viral transport media with a swab.

Oropharyngeal swab and nasopharyngeal swabs are collected using a polypropylene fiber swab or flocked swabs with plastic shafts.

a. Plasma- Centrifuging the anticoagulant blood at 1000g for 15min, and transfer supernatant to a new tube.

b. Serum - Mix collection tube by inverting 5-8 times immediately after blood collection, then let stand for 30 minutes, centrifuge at 1500g for 10min.

③ Specimen storage conditions

The collected specimens should be sent for testing immediately. Specimens should be tested within 24 hours if stored at 2°C to 8°C. Specimens that cannot be tested within 24 hours should be stored at -70°C or below (in the absence of -70°C storage conditions, specimens can be stored at -25°C to -15°C for 10 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

8 Processing and Extraction of the Sample

It is recommended to ECALBIO® Pure DNA Purification kit （Spin Column） to extract high quality DNA. The specific operation is by its instructions. The EDTA of the elution buffer may inhibit PCR amplification. Therefore, it is the best choice to elute nucleic acids with ultrapure water (Molecular Biology Grade).

Note:

The extracted DNA should be immediately tested or stored at -20℃.

The Negative Control and MPV Positive Control do not need to be extracted. The MPV Positive Control Lyophilization needs to be redissolved before use. Add 200μL MPV Negative Control to MPV Positive Control tube, mix thoroughly and transient centrifuge Then stored at - 25℃ to -15℃ after re-dissolution.

The map of the 96-well plate:

A	MPV/Endogenous
B	MPV/Endogenous
C	MPV/Endogenous
D	MPV/Endogenous
E	MPV/Endogenous
F	MPV/Endogenous
G	MPV/Endogenous
H	MPV/Endogenous

9 PCR Reaction Preparation

① Take out the 96-well plate, equilibrate to room temperature (about 15-30 minutes), and cut off the required strips based on the total number of samples (including patient specimen(s), MPV Positive Control, and MPV Negative Control). PCR tube(s) can be cut down from the strip.

② Remove the microplate sealer on the plate, then add a 25μL sample (including extracted DNA from a patient specimen(s), MPV Positive Control, and MPV Negative Control) to the PCR tube.

③ Cover the plate with the microplate sealers, mix, and transient centrifuge at 2000-6000 rpm, then put them into the PCR instrument.

10 PCR Assay

① Create a new experiment.

② Select the FAM and VIC channels.

③ Select "none" for Passive Reference (For ABI Real-Time PCR systems).

④ Set cycle conditions. Enter the reaction volume (25μL), and edit the PCR program as follows:

Step 1: 50℃ 2min;

Step 2: 95℃ 5min;

Step 3: 95℃ 10s, 60℃ 40s (fluorescence collection). (40 cycles)

Save the file. Run the program.

11 Analysis of Results

Following the completion of reactions and detection, the instrument will automatically save the results.

① Setting analysis condition: according to the image obtained from the PCR reaction, adjust the start value, end value of baseline, and threshold. The user can adjust these values according to their situation. The start value can be set at 3-15; the end value can be set at 5-20.

② Click the "Analyze" icon to update the analysis.

③ Enter the "Report" window and record unknown sample values (*Ct*) or the quantity number:

12 Interpretation of Quality Control

Assessment of clinical sample test results should be performed after the positive and negative controls have been determined to be valid. If the controls are not valid, the patient results cannot be interpreted.

The MPV Positive Control:

FAM channels has typical S-type amplification curves and Ct values ≤32. The Endogenous Control channel(VIC) have a typical S-type amplification curve.

The MPV Negative Control:

FAM and VIC channels have no Ct value or Ct value>38.

Note:

The above conditions must be met at the same time, otherwise this experiment is invalid and needs to be repeated.

The Positive Judgment Value

Through the study of reference values, it was determined that the Ct reference value of the target gene detected by this kit was 38.

13 Sample Result Judgment

① If the test sample detects a typical S-type amplification curve in the FAM channels and the *Ct* value is ⩽35, and there is a typical S-type amplification curve in the Endogenous Control channel(VIC), the sample can be judged to be MPV positive.

② If the test sample has no amplification curve or *Ct* value> 38 in the FAM channels, and there is a typical S-type amplification curve in the Endogenous Control channel(VIC), the sample can be judged to be MPV negative.

③ If the test sample yields a *Ct* value ranging from 35-38, and there is a typical S-type amplification curve in the Endogenous Control channel(VIC), the results need to retest. If the results repeated are consistent, and have a typical S-type amplification curve, the sample can be judged positive, otherwise, the sample can be judged negatively.

④ If no typical S-type amplification curve (No *Ct* value) or *Ct* value>38 is detected in the FAM and VIC channels of the test sample, it means that there is a problem with the quality of the sample or a problem with the operation. If the result is invalid, you should find and eliminate the cause, collect the sample again, and repeat the test(if the test result is still invalid, please contact the company).

14 Performance Index

① The analytical sensitivity (LoD) of the detection kit is 200 copies /mL.

② No Cross-reactivity with the human genome, Camelpox virus, Variola virus, Ectromelia virus, Vaccinia virus, Cowpox virus, Mouse hemorrhagic fever virus, Meningitis virus, Capri pox virus, Fowlpox virus, Mouse pox virus, Pigeon pox virus, Herpes simplex virus type 1&2, Varicella-zoster virus.

③ Precision:The intra-batch/ batch precision, intra-day/ day precision, and precision variation coefficients between different operators are not greater than 5%.

15 Limitations of the Procedure

① The test results of this kit are only for clinical reference. The clinical diagnosis and treatment of patients should be considered in combination with clinical observations, patient history, and epidemiological information.

② The test results may be affected by the quality of the samples collected as well as their handling, transportation and storage. Deficiencies in these factors may lead to false negative results.

③ False positive results may occur if cross-contamination is not controlled during sample processing.

④ Amplification of Endogenous Control may fail if pathogen concentration in the specimen is high.

⑤ Nucleic acid extraction reagents from other companies need to be tested on several samples to verify their suitability.

16 Precautions

① For *in vitro* diagnostic use only.

② Read instructions carefully before testing. The test must be performed according to the instructions provided.

③ Laboratory management shall be strictly by management standards of the nucleic acid test laboratory.

④ Conduct quality control for every experiment.

⑤ Pipettes used with this test must be calibrated regularly.

⑥ The entire process should be divided into three separate areas within the laboratory: The first area for reagent preparation, the second area for specimen processing and reaction system preparation and the third area for amplification.

⑦ Fluorescence detection and result analysis. The instruments, equipment, and personal protective equipment (PPE) in each area should be used independently to prevent cross-contamination.

⑧ Operators of the test should always avoid potential contamination of RNase and DNase. Test operators should not directly touch the reaction tube by

hand. Operators must use disposable gloves and testing materials without Fluorescent properties.

⑨ Follow biohazard safety standard precautions. Biological safety cabinets should be used when handling specimens to ensure the safety of operators and prevent pollution. Harmful and/or toxic specimens and reagents should be properly stored and maintained by designated personnel. Waste should be properly disposed of in special containers. Instruments and equipment such as operating tables, pipettes, centrifuges, and amplification instruments should be frequently wiped and disinfected with 10% sodium hypochlorite and/or 75% ethanol. Laboratory rooms and ultra-clean workbenches should be regularly treated with UV lamps after each experiment.

⑩ The reagents in the centrifuge tube should be fully thawed and mixed before use. Centrifuge for a few seconds to concentrate the liquid at the bottom of the centrifuge tube. When preparing the reaction system, it should be noted that the mixing of all liquids should be carried out on the vortex mixer as much as possible. Once the reaction system is completed, centrifuge for a few seconds at a low speed.

⑪ Do not mix reagents from different batches.

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