MONKEYPOX VIRUS DETECTION

The Monkeypox Virus Detection Kit is a qualitative *in vitro* real time PCR test for the detection of DNA extracted from lesion material. DNA can be extracted from acceptable specimen types including, but not limited to, lesion fluid on a dry swab, lesion fluid swab in viral transport media, lesion fluid on a slide, crust, or lesion roof. The 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. The agent detected may not be the definite cause of disease. 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.

PRINCIPLE

The purpose of this protocol is to describe the procedure used for the detection of Monkeypox virus DNA in clinical specimens by real-time PCR. This assay detects DNA at varying concentrations, providing a qualitative result of either positive, negative, or inconclusive in the identification of Monkeypox virus infections. Primer and probe are designed to detect Monkeypox genes. The assay kit also contains a positive control (Plasmid DNA) and a negative control for testing process monitoring.

REAGENTS AND MATERIALS PROVIDED

Item	Component	Quantity		Volume /T
		100 Tests / Box	1000 Tests / Box	
1	MPXV PCR Mix	1.5 mL each	15 mL each	15 µL
2	Positive Control	50 µL/ tube × 1	250 µL/ tube × 1	5 µL
3	Negative Control	50 µL/ tube × 1	250 µL/ tube × 1	5 µL

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE***), FAM and Cy5 channels.
2. Specimen Collection Containers
3. DNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex
7. High-speed centrifuge
8. Micropipette (0.5-2 µL, 1-10 µL)
9. Pipette tips with filters
10. Personal protective material

WARNING AND PRECAUTIONS

1. For use by professionals specifically trained in nucleic acid amplification techniques and *in vitro* diagnostic procedures.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should start from DNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a DNA Extraction Kit prior to testing. Do not perform a qPCR Assay directly using specimens without extraction.
9. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 12 months, and the expiry date of the product is shown on the label.

SPECIMEN TYPE and PRESERVATION

Specimen type: Lesion material.

Specimen preservation: process specimens for viral DNA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -80°C for longer storage, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

ASSAY PCR REACTION SETUP (at reagent preparation area)

A. Reagent Preparation

To calculate the number (n) of PCR reaction tubes (n= number of specimens to be tested + number of positive controls + number of negative controls), add n x15 µL of PCR Mix to a clean tube, vortex to mix well, centrifuge and aliquot 15 µL of the mix into each PCR reaction tube.

B. Addition of Viral DNA

Add 5 µL of viral DNA extracts of the specimen or positive/negative control to each PCR tube according to the reaction preparation table below for testing. Then immediately cap the tube tightly, briefly spin the mixture and move it to the amplification area for PCR detection.

PCR Reaction Preparation:

Component	Volume per test
PCR Mix	15 µL
Negative Control / Positive Control / or DNA of specimen to be tested	5 µL
Total Reaction Volume	20 µL

C. PCR amplification (at Amplification Area, use Applied Biosystems 7500 Real-Time PCR System as an example)

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimen on the Thermocycler software.
2. Enter the PCR reaction volume: 20 µL.
3. Select fluorescence detection channels: JOE, and FAM channels.
4. Select PCR cycle parameter setting following the instructions below, save the file and start the PCR run.
5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

Cycles	Step	Time	Temperature
1	Initial denaturation	2 minutes	95 ºC
40	Denaturation	10 seconds	95 ºC
40	Annealing/Extension	30 seconds	60 ºC

QUALITY CONTROL

Quality control requirements must be performed in conformance with local, accreditation requirements and the user's laboratory's standard quality control procedures.

NC (negative control) material: no obvious amplification curve for JOE and FAM detection channels.

PC (positive control) material: obvious amplification curves for JOE and FAM detection channels (Ct value ⩽37).

Pay attention that the above requirements must be met each time when the experiment is performed; otherwise, the experiment is considered as invalid. The reason of invalidation needs to be resolved and the test result needs to be repeated using residual specimens stored appropriately.

DETERMINATION OF RESULTS

1. The results can only be determined when the results of both controls meet their requirements.
2. Criterion for a positive specimen: a specimen can be reported as positive when FAM and JOE are both positive.
3. Criterion for a negative specimen: a specimen can be reported as negative when the JOE channel is positive, and the FAM channel is negative.
4. Cut off values must be determined by each laboratory during the validation phase.

Channel	Gene	Suggested results Interpretation
FAM	MPXV	Positive: If both JOE and FAM have exponential increase curves with Ct ⩽ 37. Presumptive positive: Only FAM has exponential amplification curve with Ct ⩽ 37. Negative: If FAM has a Ct >= 37 and JOE has a Ct⩽ 37.
JOE	RNase P	

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
Applied Biosystems	7500 Real-Time PCR System
	7300 plus Real-Time PCR System

PRODUCT PERFORMANCE

Product analysis performance evaluation results:

1. The analytical sensitivity of this kit is 250 copies/mL.
2. Cross-reaction: no cross-reaction with other pathogens.

LIMITATIONS OF TEST

1. This test provides a presumptive diagnosis of Monkeypox pathogen infection. Negative test results do not preclude pathogen infection. All test results should be evaluated by healthcare professionals in the context of clinical symptoms, epidemiological information, patient history, and other diagnostic test results as the basis for patient management decisions.
2. Factors that may lead to false negative results must be excluded, including poor specimen quality; specimens collected too early or too late; specimens not properly stored, transported or processed; virus variation, PCR inhibitors, etc.
3. If test results are negative but clinical symptoms persist, follow up with additional serological diagnostic testing.
4. The Assay Procedure and the Interpretation of Test Results sections must be followed closely when testing. Failure to follow the procedure may lead to inaccurate results.
5. This test should be used only with Orthopoxvirus specimens. The use of other specimen types has not been validated.
6. The quality of the sample impacts the quality of the test; improper specimen collection, storage and/or transport, and improper DNA purification may yield false negative results.
7. In some samples, extremely low levels of target (below the limit of detection) may yield an amplification signal, but results may not be reproducible.
8. Cross-contamination by samples containing high copies of Orthopoxvirus pathogens, particles or amplification products from previous reactions can yield false positive results. Take proper precautions to monitor contamination and preserve the purity of the kit component

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (858) 597-0602 or visit our website at www.biomiga.com

Symbols used

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Catalogue number
	Batch code
	The number of test
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

20220728

Version A01

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This is a Research Use Only (RUO) kit and is not to be used for diagnostic purposes of any kind without validation.