For Medical Professional Use Only

Real time PCR Kit for Monkeypox Virus

INSTRUCTION FOR USE

Product Name

Real time PCR Kit for Monkeypox Virus

Product Description

This kit is used for in vitro qualitative nucleic acid detection of Monkeypox Virus in Human serum, lesion exudate samples and scab specimens. Primer sets and FAM labeled probes are designed for specific detection of Monkeypox Virus, Human RNase P gene extracted concurrently with the test sample provides an internal control to validate nucleic extraction procedure and reagent integrity. Probe targeting human RNase P gene is labeled with VIC.

Size

48 Tests/kit, 96 Tests/kit

Kit Contents

Components	48 Tests/kit	96 Tests/kit
PCR reaction buffer	672 μL × 1 tube	672 μL × 2 tube
PCR enzyme mix	50 μL × 1 tube	100 μL × 1 tube
Positive control	100 μL × 1 tube	200 μL × 1 tube
Negative control	100 μL × 1 tube	200 μL × 1 tube

Precautions:

1. Components with different lot numbers cannot be used together.

2. User prepared Nucleic Acid extraction Kit.

Storage and Expiration

This kit expires in 12 months when stored at -20±5℃ and in prevention of light.

Avoid repeated freeze-thaw cycles (5 times maximum).

Manufacture date and expiration date are printed on the label.

Applicable Instruments

Real-Time PCR System: Molarray MA-6000, ABI 7500, ViiA™ 7, QuantStudio 5, QuantStudio 6/7 pro, QuantStudio 6/7 flex, Agilent Mx3000P/3005P, Rotor-Gene™ 6000/Q, Bio-Rad CFX96 Touch™/iQ™ 5，Hongshi SLAN-96S/96P，AGS8830, AGS4800

Specimen Handling and Storage

1. Acceptable Specimens: Human serum, lesion exudate samples and scab specimens.

2. Collect samples in sterile tubes.

3. Contamination should be avoided during collection, storage and transportation of the samples.

4. Specimens can be stored at 2~8℃ for up to 24 hours after collection. If a delay in extraction is expected, store specimens at -70℃ or lower. Avoid repeated freeze-thaw cycle of the sample and make sure sample is completely thawed before extraction.

5. Transport samples in sealed box with dry ice or ice bag.

Protocol

Precaution: All the reagents should be thawed completely before use, then vertex and spin down at 6,000rpm.

1. Sample treatment (Sample treatment zone)

Extract nucleic acid with viral nucleic acid extraction kit. nucleic acid extracted can be stored at -70℃ or lower for 6 months. Positive control and negative control can be used without extraction.

2. Preparation of amplification reagents (Mix preparation zone)

Take N (N=negative control number + nucleic acid sample number +positive control number) PCR tubes, add 14 μL PCR reaction buffer and 1 μL PCR enzyme mix to each tube.

Components	Volume per test
PCR reaction buffer	14 μL
PCR enzyme mix	1 μL

Centrifuge the PCR tubes at 6,000rpm for 30s and transport to sample treatment zone.

3. Sample loading (Sample treatment zone)

Add 10 μL nucleic acid sample, negative control and positive control to the above PCR tubes respectively. Cap the tubes tightly and centrifuge at 6,000rpm for 10s and then transport to PCR amplification zone.

* Precaution: Adding samples in the following order is recommended: negative control-> nucleic acid sample -> positive control.

4. PCR amplification (PCR amplification zone).

4.1. Put the caped PCR tubes into real-time PCR machine for amplification.

4.2. Thermal cycling setting

Steps	Temperature	Duration	Cycle
1	50℃	2min	1
2	95℃	2s	1
3	95℃	1s	41
	60℃	13s / 20s /35s	

Collect fluorescent signals at step 3:60℃; 35s for ABI 7500, 20s for SLAN-96S/96P, while 13s for other Real-Time PCR Systems. The total volume: 25 μL.

NOTE: for ABI7500, ViiA™ 7, QuantStudio series instruments, choose ‘none’ as both passive reference and quencher.

4.3. Disposal after detection

Dispose the PCR tubes in a sealed bag after reaction and treat the used tubes as medical wastes.

5. Settings for result analysis

Set the baseline at a region before the exponential amplification where the fluorescent signals of all the samples are relatively stable (no significant fluctuations in all the samples); set the starting point (cycle number) away from the signal fluctuations at the starting phase of fluorescence collection; set the end point (cycle number) 1~3 cycles before the Ct of the first sample to enter exponential amplification. 4~15 cycles are recommended.

Set the threshold right above the highest point of the negative control amplification curve (irregular noise curve).

6. Quality control criteria

Prior to evaluating the specimen results, the Positive Control and Negative Control should be interpreted using the interpretation table below, and the Positive Control and Negative Control curve must be performed correctly, otherwise the sample result is invalid.

Channels / Controls	Cycle threshold（Ct）value	
	FAM	VIC
Negative control	Ct > 40 or UNDET	Ct > 40 or UNDET
Positive control	Ct ≤ 35	Ct ≤ 35

Result Determination

FAM channels for Monkeypox Virus, detection result should be interpreted as below.

1. Positive: Ct ≤ 38 and amplification curve is S-shaped.

2. Suspected: 38 < Ct ≤ 40 and amplification curve is S-shaped, a second test is needed. Consider positive if Ct ≤ 40 and amplification curve is S-shaped for the second test. Considered negative if Ct > 40 or null Ct and Ct ≤ 35 in VIC channel for the second test.

3. Negative: Ct > 40 or null Ct and Ct ≤ 35 in VIC channel.

4. Re-test: Ct > 40 or null Ct and Ct > 35 in VIC channel.

Performance Index

1. Sensitivity: 200 copies/mL.

2. Specificity: No cross reaction with Enterovirus(EV), Measles virus(MV), Rubella virus(RV), Varicella-zoster virus(VZV), Dengue virus(DenV), Human Parvovirus B19(HPVB19), Epstein-barr virus (EBv), human herpes virus 6(HHV-6)

3. Precision: CV ≤ 5%.

Limitations

1. Negative results do not preclude infection with Monkeypox Virus and should not be the sole basis of a patient treatment decision.
2. Reliable results are dependent on the adequate specimen collection, proper transportation storage and processing procedures.
3. Inhibitors present in the sample and/or errors in the following assay procedure may lead to false negative results.
4. A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
5. Potential mutations within the target regions of the virus genome covered by the test primers and/or probes may result in failure to detect the presence of the pathogens.
6. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified products, or from non-specific signals in the assay.

Precautions

1. Management of the lab should comply with national regulations on gene amplification testing labs.
2. This kit is used for *in vitro* diagnosis only. It is for medical professional use only.
3. To prevent contamination, the same item should not be used cross different zones. Clean the bench immediately after conducting experiments.
4. Reagents of the kit should be thoroughly thawed and centrifuge briefly before use.
5. Reaction tubes containing reaction aliquots should be capped or placed in sealed bags before being delivered to the sample zone.
6. When loading sample, pipette the sample directly into the reaction solution without contacting the tube wall. Cap the tube immediately after loading the sample.
7. After the amplification is completed, the reaction tubes should be taken out immediately, placed in designated sealed bags and disposed at designated locations.
8. Avoid generating bubbles when aliquoting reaction mixture. Ensure the reaction tubes are capped before loading them into the PCR machine to avoid contamination.
9. Pipette tips used in the experiments should be directly tossed into a waste tank containing 1% sodium hypochlorite and disposed together with other wastes after sterilization.
10. Disinfect the working benches and other items regularly with 1% sodium hypochlorite, 75% alcohol or ultraviolet lamp.

Company Information

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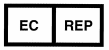
SYMBOL	DESCRIPTION	SYMBOL	DESCRIPTION
	Manufactured By	REF	Catalog Number
	Authorized Representative		Potential Biological Hazards After Use
	InVitro Diagnostic Medical Device		Do Not Reuse
	Batch Code		Date of Manufacture
	Expiration Date in Year-Month-Day Format		Consult instructions for use
	Temperature Limitation		Keep away from sunlight
	caution		