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 ä^c&ç} Á-Á []\^} [cÁkã`•ÁÁÁ^ { Á:Á^•á } Á`c` äæ`Áæ]|^•.
 The kit contains a specific ready-to-use system for Monkeypox Virus detection through Reverse Transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system.

2. Intended Use

The Monkeypox Virus real time PCR kit is a test for the detection of Monkeypox Virus in serum or lesion exudate samples in real time PCR systems.

3. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Product Description

Monkeypox virus is the virus that causes the disease monkeypox in both humans and animals. Monkeypox virus is an Orthopox virus, a genus of the family Poxviridae that contains other viral species that target mammals. The virus is mainly found in tropical rainforest regions of central and West Africa. The primary route of infection is thought to be contact with the infected animals or their bodily fluids. The genome is not segmented and contains a single molecule of linear double-stranded DNA, 185000 nucleotides long.

The Monkeypox Virus real time PCR Kit contains a specific ready-to-use system for the detection of the Monkeypox Virus through polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the Monkeypox Virus DNA. Fluorescence is emitted and measured by the real time systems' optical unit during the PCR. The detection of amplified Monkeypox Virus DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and serum or lesion exudate samples are used for the extraction of the DNA. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control defined as 1x10^7 copies/ml is supplied which allow the determination of the gene load. For further information, please refer to **section 10.3 Quantitation**.

5. Kit Contents

Ref.	Type of reagent	Presentation 25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml
2	MPV Reaction Mix	1 vial, 950µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control (IC)	1 vial, 30µl

6	MPV Positive Control(1x10^7 copies/ml)	1 vial, 30µl
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Analysis sensitivity: 5x10^3 copies/ml;

LOQ: 1x10^4~1x10^8 copies/ml.

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

6. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super mix should be stored in the dark.

7. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "ependorf" type tubes (RCF max. 16,000 x g)

8. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

9. Sample Collection, Storage and transportation

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local

regulations for the transport of etiologic agents.

10. Procedure

10.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. It's better to use commercial kits for nucleic acid extraction.

- 1) Pipet 50µl sample (serum, or lesion exudates dissolved in 1ml saline) to a 0.5ml tube, add 50µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
- 2) Incubation the tube for 10 minutes at 100°C.
- 3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can used for the PCR template.

10.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.

Add the internal control (IC) 1µl/rxn and the result will be shown in the HEX/VIC/JOE.

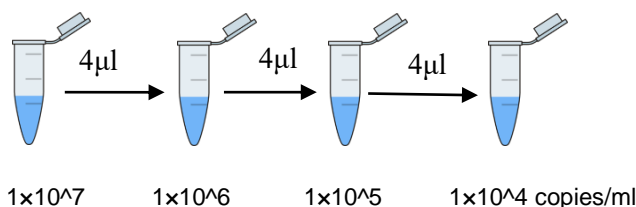
10.3 Quantitation

The kit can be used for **quantitative or qualitative** real-time RT-PCR.

For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows. Molecular Grade Water is used for dilution.

The step of dilution is not needed for performance of qualitative real-time PCR.

Take positive control (1x10⁷ copies/ml) as the starting high standard in the first tube. Respectively pipette **36µl** of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



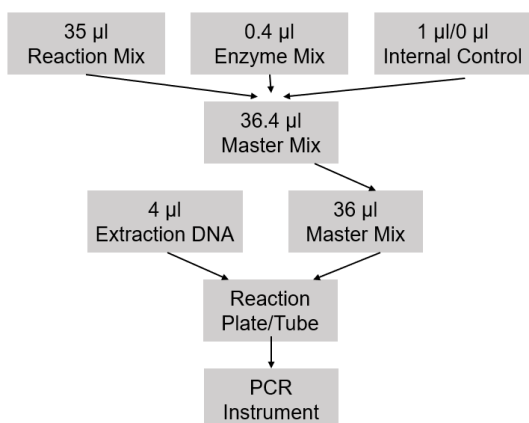
To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

Attention:

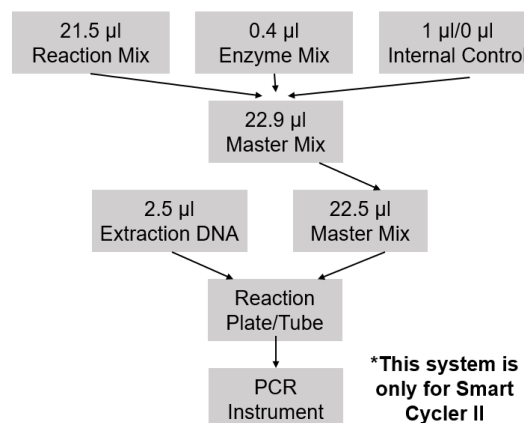
- A. Mix thoroughly before next transfer.
- B. The positive control (1x10⁷ IU/ml) contains high concentration of the target DNA. Therefore, be careful during the dilution in order to avoid contamination.

10.4 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



OR



*This system is only for SmartCycler II

※PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

- 1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
- 2) Pipet **36µl (22.5µl for SmartCycler II)** Master Mix with micropipets of sterile filter tips to each of the Real time PCR reaction plate/tubes. Separately add **4µl (2.5µl for SmartCycler II)** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
- 3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- 4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 5sec, 60°C for 30sec (Fluorescence measured at 60°C)	40cycles
Selection of fluorescence channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	IC

5) If you use ABI Prism® system, please choose "none" as **passive reference** and **quencher**.

11.Threshold setting: just above the maximum level of molecular grade water

12.Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

13. Quality control: Negative control, Positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid.

Control	Ct value	
	FAM	HEX/VIC/JOE
Molecular Grade Water	Blank	25~35
Positive Control (qualitative assay)	≤35	---
QS (quantitative detection)	Correlation coefficient of QS curves ≤ -0.98	

14. Data Analysis and Interpretation: The following sample results are possible:

	Ct value		Result Analysis
	FAM	HEX/VIC/JOE	
1#	Blank	25-35	Below the detection limit or negative
2#	≤38	---	Positive; and the software displays the quantitative value
3#	38-40	25-35	Re-test; If it is still 38~40, report as 1#
4#	Blank	Blank	PCR Inhibition; No diagnosis can be concluded.

For use with ABI Prism® 7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II; Bio-Rad CFX 96; Rotor Gene™ 6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler®480 Instrument

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