Monkeypox Virus Detection Kit

CE IVD

For in vitro diagnostic use only.
For laboratory and professional use only.

Cat. No: LMO-01-24

Manual Version: 100522-1
Approval Date for Use:10.05.2022

Tablo 1. Kit Content

Storage Temperature: -20°C, Transport Temperature: +2-8°C		
Kit Content	**Quantity (10 µL Reactions)** **24 Rxns**	**Consumption / Reaction**
MP-Oligo Mix: • Monkeypox (I4L, H5R, I6L) (FAM - Green) • Internal Control (IC) (RPP30) (HEX or VIC - Yellow)	1 x 60 µL	2.5 µL
MP-2X Prime Script Mix • DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase and ribonuclease inhibitor	1 x 60 µL	2.5 µL
NTC (Negative Template Control) Test it in each run for contamination control	1 x 120 µL	5 µL
MP-PC: • Positive Control Test it in each run for reactive stability control	1 x 120 µL	5 µL
Instruments and equipments supplied by the user		
1) Real-Time PCR Instrument: FAM/HEX channel, Ramp rate ≥3 °C/sec. **2)** 1-10 µL, 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips (DNase and RNase free) **3)** Quick Spin Centrifuge: min. 3000 rpm **4)** Vortex **5)** Nuclease-free water/Viral Transport Medium/Serum physiologic	**6)** 1.5 or 2 mL microcentrifuge tubes **7)** Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume, **8)** UV Cabinet for PCR Setup **9)** Cold Tube Rack (for microcentrifuge tubes and PCR tubes/strips) **10)** Disposable powder-free nitrile gloves	

Intended Use and Test Principle

The Laborant Monkeypox Virus Detection Kit is used to detect all variants of MPV (Monkeypox Virus) that cause Monkeypox. Monkeypox virus is in the Orthopox family of viruses, which also includes the variola viruses that cause smallpox. It has similar symptoms to Smallpox but is milder and rarely fatal. Monkeypox disease is not associated with chickenpox (chickenpox).

The kit allows to achieve RT-qPCR result in around 34 minutes and may vary according to the Ramp Rate of the device. The study material is nucleic acid isolates obtained from lesion swab samples taken in the VTM. It detects the I4L, I6L and H5R gene regions of the Monkeypox virus in the FAM channel by real-time PCR (qPCR) (RT-qPCR).

The human RPP30 gene region is amplified and detected in the HEX (VIC) channel for internal control for sample collection, nucleic acid isolation, and detection of potential reaction inhibitory problems.

Analytical Specifications

The kit is validated with SI-NAT Extraction Consumables (SI-NAT Viral Nucleic Acid Buffer Cat No: SI-NAT-100). and for 10 and 20 µL qPCR volumes by using Himedia Insta Q96™, Bio-Rad CFX96 Touch™, Qiagen Rotor- Gene® 5 Plex and Applied Biosystems QuantStudio™ 5 Real-Time PCR systems. The LOD of the kit is 1,000 copies/mL for the Monkeypox I4L, I6L and H5R regions. The exclusivity tests of the kit were tested in-silico for 25 different viral and bacterial strains. In-silico tests have shown that the kit may cross-react in sub-variants of some Orthopox species.

Collection, Storage and Shipment of Clinical Specimens

Swab samples should be collected by using Dacron or Polyester swabs. Other specimen types should be transferred in sterile containers. In the transport phase, Viral Transport Medium (VTM) (Preperation of viral transport medium, Center for Disease Control and Prevention, SOP#: DSR-052-01) or Laborant® SI-NAT Viral Transfer Tubes (Cat No:SI- NAT-100). Samples collected into SI-NAT Tubes should be stored and transported at 2-8 °C or room temperature until they arrive at the laboratory. Samples should not be kept at room temperature for more than 24 hours. It can be stored up to 21 days at +2-8 °C. Samples should never be frozen.

DNA isolation should be done with the help of a commercial kit from the samples reaching the laboratory. A pure sample will improve result quality. Laborant SI-NAT Viral Transfer Tube provides DNA extraction due to the chemicals it contains.

Warnings

1. The kit should be stored away from nucleic acid sources and qPCR amplicons.
2. The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers.
3. Master stock reagents should be kept on the cold block during the PCR setup; if possible, the PCR setup should be performed on the cold block.
4. Kit components should be mixed by gently shaking before use.
5. The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate.
6. Template nucleic acid and positive control tubes should always be kept closed, except for fluid transfers.
7. The wipeable surfaces of the rooms, benches and devices where the test is performed should be cleaned regularly with 10% bleach (NaClO). Alcohol and its derivatives should not be used as disinfectants.
8. The qPCR completed reaction tubes should be disposed of before opening in the laboratory.

RT-qPCR Application Protocol

Before starting the assay, please consider the following:

- The kit was validated only for the template nucleic acid volume that is 25% of the total qPCR volume.
- The kit can not be used with real-time PCR instruments without the periodic maintenance records.
- Only white 0.1 mL qPCR plates/strips/tubes can be used for the assay. The caps must be clear and transparent.
- 0.1 ml and 0.2 ml clear qPCR tubes can be used for the assay, while slightly better performance can be obtained using the 0.1 ml tubes for Rotor Gene Q instrument.

Edit the program to the qPCR device as follows and add the reagents to the qPCR tubes in the order specified below, close the tubes, place them into the qPCR device and start the run (Table 2).

Table 2. Reaction set-up and qPCR program details

Reaction setup			qPCR Program		
Component	**Reaction**		**Cycle**	**Temperature**	**Duration**
MP-2X Prime Script Mix	2.5 µL	5 µL	1	52 °C	1 min
MP-Oligo Mix	2.5 µL	5 µL	1	95 °C	10 sec
			40	95 °C	1 sec
Template Nucleic Acid	5 µL	10 µL		55 °C	1 sec
Total Reaction Volume	10 µL	20 µL		FAM-Green / HEX-Yellow Read	

Interpretation of The Assay Results

The recommended threshold level to calculate the number of threshold cycles (Cq) for both 10 µL and 20 µL reactions is 200 RFU for Bio-Rad CFX96 Touch™ and Himedia Insta Q96™. In Rotor-Gene® instruments, the sigmoidality of the amplification curves should be evaluated from the "Raw Data" screen. To see the Ct values of sigmoidal curves in Rotor-Gene® devices; on the analysis screen, "Dynamic Tube" should be active, "Slope Correct" options should be passive, "Outlier Removal" option should be "0", the threshold level should be set to 0.02. Shape of the amplification curves obtained in the FAM/HEX channels are examined and non-sigmoidal curves are recorded as negative. The result is recorded as positive if Cq<35 and the analysis result should be interpreted according to Table 3.

Table 3. Interpretation of Patient Samples

Cases	FAM (Green)	HEX (Yellow)	Comment
1	-	+	Negative
2	+	+ or -	Monkeypox Positive
3	-	-	Test İnvalid, Repeat
4	+ (Cq≥35)	+ or -	Low Viral Load , Repeat

Limitations

- The performance of the Laborant Monkeypox Virus Detection Kit has been determined only for DNA sample obtained from skin lesion swab samples collected into the VTM.
- Mutations within the target regions of the Monkeypox Virus Detection Kit could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled.
- Inhibitors or other external factors may cause false negative results. False negative results can also occur in case of low viral load.
- Results may be affected by patient factors (e.g., presence of symptoms), and/or stage of infection.