# MBPCR269 Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit

## Description

Monkeypox is a viral zoonotic disease caused by the monkeypox virus, which belongs to the Orthopoxvirus genus in the Poxviridae family. Transmission to humans typically occurs through direct contact with infected animals or humans, or exposure to contaminated materials. Although first identified in 1970 in the Democratic Republic of Congo, monkeypox has historically been reported mainly in rural and rainforest regions of Central and West Africa. In recent years, the disease has attracted global attention due to several outbreaks. Notably, a significant outbreak began in 2022, with a marked increase in cases across several countries outside Africa. By mid-2023, the World Health Organization (WHO) reported that over 80,000 cases had been confirmed globally, with a substantial number of cases occurring in previously non-endemic regions. As of early September 2024, the WHO reported a total of approximately 95,000 confirmed cases worldwide since the beginning of the 2022 outbreak. The most affected regions include the Americas and Europe, with a notable increase in cases reported in the United States, Brazil, and several European countries. The outbreak has led to several fatalities, though the majority of cases have been mild or moderate.

Effective surveillance, precise diagnosis, and prompt management are essential for controlling monkeypox outbreaks. Recent advancements in molecular diagnostics have greatly enhanced the detection and management of this virus. Real-time PCR assays, which are considered the gold standard for rapid diagnosis, have seen innovations such as multiplex assays that can distinguish monkeypox from other orthopoxviruses. The Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit exemplifies these advancements, offering a streamlined approach for rapid diagnosis of monkeypox in a single tube.

**NOTE**: Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit is for *in-vitro* use only.

## Intended Use

Hi-PCR ® Monkeypox Virus Multiplex Probe PCR Kit is used for the sensitive and specific detection of Monkeypox virus in clinical samples. The clinical samples may include skin lesion material, lesion surface and/ or exudate, roof of one lesion, lesion crusts, oropharyngeal swabs that aid to diagnosis. This ready-to-use real-time PCR system employs hydrolysis probes, which enable accurate differentiation of Monkeypox virus from other orthopoxviruses. The kit can detect both Clade I and Clade II of MPXV, though it does not differentiate between these two clades. The kit is intended for use by qualified clinical laboratory personnel trained in the techniques of real-time PCR and in-vitro diagnostic procedures.

## Principle

The Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit is a ready-to-use real-time PCR system designed for accurate detection of Monkeypox virus and orthopoxvirus DNA. It utilizes hydrolysis probes—short oligonucleotides with a fluorescent dye and a quencher dye. During amplification, the probe is cleaved by DNA polymerase, separating the dyes and increasing fluorescence, which is monitored in real-time for precise detection. This kit features primers and probes targeting specific regions of the Monkeypox virus (MPXV) and a generic orthopoxvirus gene (POPV), along with a human internal control (IC) to ensure test reliability. Detection is facilitated through three fluorescent channels: FAM for MPXV, JOE for POPV, and Texas Red for internal control.



ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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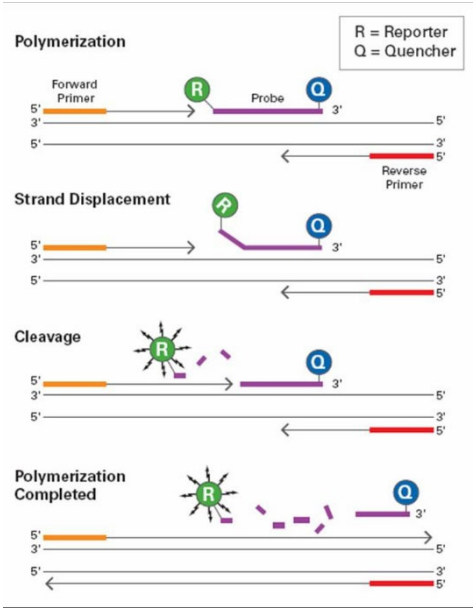
**Controls**

**Internal control (IC):** An endogenous internal control is a DNA sequence that is naturally present in the test samples, is amplified in the same tube with a different set of primers and probe which may aid in identification of possible PCR inhibition, DNA purification efficiency and assessing the quality of the sample.

**Positive control (PC):** A positive control mimics a sample which contains all the target DNA sequences that the PCR is designed to amplify. It is included in a PCR assay to check the proper and intended functioning of all the PCR reagents.

**No template control (NTC):** A No Template Control is a PCR reaction that contains all the PCR reagents but does not include any DNA template. PCR grade water is used as the template to confirm that any observed amplification in the test samples is not due to contamination or non-specific amplification.

**Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR**




**Polymerization: A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively**

**Strand displacement: When the probe is intact, the report dye emission is quenched.**

**Cleavage: During each extension cycle,the DNA polymerae cleaves the reporter dye from the probe**

**Polymerization completed: Once separated from the quencher, the reporter dye emits its characteristic fluorescence**

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e. the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'- nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, inclusion of the probe does not inhibit the overall PCR process.

## Features

**Molecular Features:**

- Accurate detection of Monkeypox virus (MPXV) [Clade I and Clade II] and orthopoxvirus DNA in clinical samples in a single assay.
- High sensitivity – 3 copies/µL for the MPXV and 1 copy/µL for the orthopoxvirus.
- High specificity – No cross reactivity of MPXV with other common respiratory pathogens.

**Technology features:**

- Fast and reliable results within 90 minutes.
- Includes all reagents & controls for validity of the test.
- Open system – Compatible with 3-channel, 4-channel and 5-channel qPCR cyclers.
- Wet-lab assays validated on the Bio-Rad CFX Opus 96, Applied Biosystems QuantStudio 5 and Insta Q96® Plus Real Time PCR Systems.

**Types of Specimens:** Recommended specimen type for laboratory confirmation of monkeypox is DNA extracted from skin lesion material, including swabs of lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts. Swab the lesion vigorously, to ensure adequate viral DNA is collected. Both dry swabs and swabs placed in viral transport media (VTM) can be used. In addition to a lesion specimen, the collection of an oropharyngeal swab is encouraged. Specimens collected for MPXV investigation should be refrigerated (2 to 8°C) or frozen (-20°C or lower) within one hour after collection. If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20°C or lower. Longer term specimen storage (>60 days from collection) is recommended at -70°C (World Health Organization. (2022). Laboratory testing for the monkeypox virus: interim guidance, 23 May 2022. World Health Organization. https://apps.who.int/iris/handle/10665/354488).

**Specimen Handling**

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids. Safety guidelines may be referred to in individual safety data sheets.

**Storage and Shelf life**

The kit provided has a shelf-life of 12 months when stored between -10°C and -20°C. Repeated thawing and freezing of PCR reagents should be avoided, not more than 5 freeze-thaw cycles, as this may reduce the sensitivity. If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze-thaw cycle. Exposure to light, heat or humidity may also affect the shelf life of some of the kit components and should be avoided. Degradation of specimen/ extracted DNA can also hamper the sensitivity of the assay. HiMedia Laboratories does not recommend using the kit after the expiry date stated on package.

**Kit Contents:** The provided PCR kit contains:

| Components | Product code | Reagents provided for (reactions)* (µL) | | |
|---|---|---|---|---|
| | | 25R | 50R | 100R |
| Monkeypox Master Mix | DS2071 | 135 | 270 | 530 |
| Monkeypox Primer-Probe Mix | DS1288 | 41 | 81 | 156 |
| Monkeypox Positive Control | DS1291 | 51 | 102 | 187 |
| Water | DS0440 | 186 | 372 | 717 |

***For 20 µL PCR reaction**

**Materials needed but not provided:** All materials are available through www.himedialabs.com

| Product name | Product Code |
|---|---|
| **Real-Time PCR Instrument and equipment** | |
| Insta Q96®AG Real time PCR System, 96 well block, 5 channels | MBLA027 |
| Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels | MBLA028 |
| Insta Q96® Plus Real time PCR System, 96 well block, 5 channels | LA1073 |
| Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels | LA1074 |
| Insta Q96® Real time PCR System, 96 well block, 5 channels | LA1012 |

| Nucleic acid extraction system and associated materials | |
|---|---|
| HiPurA® Pre- filled Cartridges for Viral Nucleic Acid Purification | MB582PC16 |
| HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification | MB582MPF16 |
| HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification | MB582MPF-32 |
| HiPurA® Pre- filled Plates for Viral Nucleic Acid Purification [For Insta NX® Mag96] | MB582MPF-96 |
| Insta NX® Mag16, Insta NX® Mag16$^{Plus}$ | LA1118, MBLA018 |
| Insta NX® Mag32, Insta NX® Mag32$^{Plus}$ | LA1096, MBLA019 |
| Insta NX® Mag96 | LA1097 |
| HiPurA® Viral DNA/RNA Purification Kit | MB582 |
| TabSpin™ Microcentrifuge | LA1089/LA1090 |
| **Tubes, plates, and other consumables** | |
| Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL) | LA611/LA614/LA615 |
| µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL) | LA955/LA958/LA959/LA960 |
| Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL) | MBLA009/MBLA011/MBLA008 |
| Barrier Tips, Maximum capacity 10 µL | LA749A |
| Barrier Tips, Maximum capacity 200 µL | LA751A |
| Barrier Tips, Maximum capacity 1000 µL | LA859A |
| 8-strip tubes & optically clear flat caps for PCR | PR17, PR22, PR23 |
| PCR Tubes, 0.1mL, 0.2 mL; PCR Plates | PW1255/PR2/PR3/PR19 |
| Optical Sealing film | PR18 |

**Kit Compatibility with Real-Time PCR systems:**

Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit compatible to:

| Real-Time PCR system | Company | Dye 1 (MPXV) | Dye 2 (POPV) | Dye 3 (Texas Red) |
|---|---|---|---|---|
| Insta Q96®AG/ Insta Q96®AG 6.0/Insta Q96® - 6.0/Insta Q96® Plus/Insta Q48® M4 | HiMedia Laboratories Pvt. Ltd. | FAM | JOE | Texas Red |
| QuantStudio™ 5 | Applied Biosystems | FAM | JOE/HEX/VIC | Texas Red/ ROX |
| ABI® Prism SDS 7500 | Applied Biosystems | FAM | JOE/HEX/VIC | Texas Red/ ROX |
| BioRad CFX Opus 96/ CFX96 Touch/ CFX384 Touch | Bio-Rad Laboratories, Inc. | FAM | JOE/HEX | Texas Red |
| Rotor-Gene® Q/ Corbett Rotor-Gene® 6000 | QIAGEN | Green | Yellow | Orange |
| QIAquant 96 & 384 5plex | QIAGEN | FAM | JOE/HEX | Texas Red |
| Roche LightCycler® 96 / LightCycler® 480 | Roche | FAM | JOE/HEX/VIC | ROX/ Texas Red |
| AriaMx | Agilent | FAM | HEX | ROX |
| Alta RT-96E/96S | Athenese-Dx Private Limited | FAM | VIC/ HEX/ TET/ JOE | ROX/ Texas Red |
| qTOWER$^3$ | Analytik Jena | FAM | JOE/HEX/VIC | ROX/ Texas Red |

**Note: Ensure that the Real-Time PCR system is calibrated for dyes and is maintained according to the manufactureŕ s instructions and recommendations.**

**General Preparation Instructions**

- Before using all PCR, components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

**Protocol for PCR Master Mix Preparation**

1. In the "Master mix Preparation" area, thaw all components from the kit on ice, mix by inverting the tubes and centrifuge the reagents for several seconds. Keep on ice for later use.
2. Based on the number of specimens to be tested (N), including the PC and NTC, calculate the volume of the components to be added as N* volume of 1X

| Components | Product Code | Volume (µL) to be added for 1R (for a 20 µL reaction) |
|---|---|---|
| Monkeypox Master Mix | DS2071 | 5.0 |
| Water | DS0440 | 5.0 |
| Monkeypox Primer-Probe Mix (PPM) | DS1288 | 1.5 |
| Monkeypox Positive Control / Viral Template DNA/Water | DS1291 | 8.5 |
| Total volume | - | 20 |

3. Use 1.5 mL Nuclease free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.
4. Load 11.5 µL of master mixture (Monkeypox Master Mix + Water + Monekypox PPM) into the 0.1/0.2 mL PCR reaction plate/strips, compatible to the instrument to be used.
5. In the "Nucleic Acid Handling" section, add 8.5 µL of Monkeypox Positive Control or Viral Template DNA to the plate or strip. For the negative template control, add 8.5 µL of water.
6. Tightly cap the strips or seal the plate using an optically clear adhesive film.
7. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube.
8. Place the plate/strips in Real-time PCR machine and set the PCR program.

**B. Recommended PCR program**

| | | | | |
|---|---|---|---|---|
| 1. | Initial denaturation | : | 95°C for 10 minutes | |
| 2. | Denaturation | : | 95°C for 15 seconds | No. of cycles: 45 |
| 3. | Annealing | : | 55°C for 40 seconds (Sampling) | |
| | Sampling | : | FAM/JOE/Texas Red | |

**C. Data Analysis**

The following conditions should be met for a valid diagnostic test:

| Control | Detection channel | | |
|---|---|---|---|
| | FAM (MPXV) | JOE (POPV) | Texas Red (Internal Control) |
| **Monkeypox Positive Control** | + | + | + |
| **Negative Template Control** | - | - | - |

**D. Data Interpretation**

| Detection Channel | | | Result Interpretation |
|---|---|---|---|
| **FAM (MPXV)** | **JOE (POPV)** | **Texas Red (Internal Control)** | |
| + | + | +/-* | Positive for Monkeypox Virus |
| - | + | +/-* | Negative for Monkeypox Virus but positive for Orthopoxvirus |
| - | - | + | Negative for Monkeypox Virus and Orthopoxvirus |
| - | - | - | Inconclusive test** Likely poor extraction or sample quality. PCR inhibition or reagent failure. |

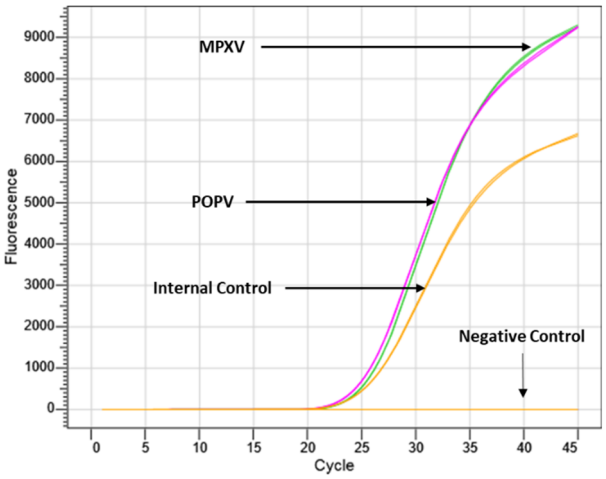
*The presence or absence of a signal in the Texas Red channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

**When an inconclusive result is obtained, repeat PCR or re-test the extracted DNA or re-extract the specimen and test the newly extracted DNA.

| Ct value | Result |
|---|---|
| ≤ 35 | Detected (+) |
| > 35 or N/A | Not detected (-) |

**Note:** Data on the accuracy of specimen type recommended for diagnosis is limited for monkeypox virus detection, therefore a negative throat swab specimen should be interpreted with caution.

**Amplification Data**




| Sr. No. | Sample | Ct value |
|---|---|---|
| 1. | MPXV | 25.59 |
| 2. | POPV | 25.56 |
| 3. | Internal Control | 25.21 |
| 4. | Negative Template Control | - |

**Image representing probe based real-time amplification data of Monkeypox Virus with $C_t$ values (provided in table). The results completely depend upon sample types.**

## Performance Evaluation

### Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity of the Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit was established as the lowest concentration of target DNA that can be reliably detected with 95% confidence. This sensitivity assessment was conducted using synthetic DNA, where a 10-fold dilution series was tested in triplicate for each concentration. The preliminary limit of detection (LoD) was then confirmed by performing 20 replicates at the concentration identified as the LoD. The results demonstrated that the Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit can detect the Monkeypox virus (MPXV) at a

sensitivity of 3 copies/µL and the orthopoxvirus gene (POPV) at 1 copy/µL, with a confidence level of 95% or greater. Therefore, the LoD is established as 3 copies/µL (or 3000 copies/mL) for the MPXV gene and 1 copy/µL (or 1000 copies/mL) for the POPV gene.

**Inclusivity - Analytical Sensitivity**

An *in-silico* analysis was performed to evaluate the inclusivity of the Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit. This involved mapping the primers and probes against 7,950 sequences available in GenBank at NCBI, of which only 6,525 sequences with genome sizes exceeding 195,000 bp (mostly complete genomes) were included. Among these, 6,222 sequences (95.35%) demonstrated complete homology with the forward primer and the probe. The reverse primer exhibited a single mismatch in the majority of evaluated clade IIb monkeypox virus strains; however, this single mismatch is not expected to affect test performance. Overall, the reverse primer showed over 95% homology in 6,329 sequences (97.0%). The table below lists representative sequences from both clades that maintain >95% homology across all oligonucleotides in the kit.

| Genbank Accession | Name | Genbank Accession | Name |
|---|---|---|---|
| **Monkeypox virus Clade Ia** | | | |
| KJ642618.1 | Cameroon-1990 | MN702448.1 | 015c_contig_SPADES |
| KJ642619.1 | Gabon 1988 | KJ642618.1 | Cameroon-1990 |
| OP498046.1 | BNITM-Gabon1988 | MN702451.1 | A6_contig_SPADES |
| KJ642613.1 | Congo_8 | PP601183.1 | 23MPX1766C |
| DQ011154.1 | Congo_2003_358 | JX878411.1 | DRC_06–1074 |
| JX878422.1 | DRC_07–0286 | KJ642612.1 | Ikubi |
| JX878420.1 | DRC 07–0283 | HM172544.1 | Zaire 1979–005 |
| **Monkeypox virus Clade Ib** | | | |
| JX878417.1 | DRC 07–0104 | PQ305788.1 | 38_DRC_HGRK_SouthKivu_2023 |
| PQ281491.1 | Monkeypox virus/Human/Thailand/DDCBIDI-002/2024 | PQ281490.1 | Monkeypox virus/human/Thailand/DDCBIDI-001/2024 |
| PQ240043.1 | CU-ID24110 | PP601220.1 | 24MPX0226V |
| PP601228.1 | RDC-NKV-GOM-MPOX-010 | PQ305781.1 | 30_DRC_HGRK_SouthKivu_2024 |
| PQ178862.1 | KEM/0010724 | PQ305770.1 | 16_DRC_HGRK_SouthKivu_2024 |
| PQ305814.1 | 79_DRC_HGRK_SouthKivu_2024 | PQ305768.1 | 14_DRC_HGRK_SouthKivu_2024 |
| **Monkeypox virus Clade IIa** | | | |
| DQ011156.1 | Liberia_1970_184 | KJ136820.1 | Ivory Coast 2012 |
| KP849470.1 | Cote d'Ivoire_1971 | AY753185.1 | COP-58 |
| DQ011157.1 | USA/Ghana_2003 | OP587264.1 | MPXV/Human/USA/CA-CDPH-MPX000040/2022 |
| DQ011157.1 | USA_2003_039 | | |
| **Monkeypox virus Clade IIb** | | | |
| PP852957.1 | VSP199 | PP852957.1 | VSP191 |
| PP860029.1 | 22V-09327 | PP860026.1 | 14V-4552 |
| PP860030.1 | 22V-07739 | | |
| **Monkeypox virus Clade IIb Lineage A** | | | |
| MT903338.1 | MPXV-M2957_Lagos | OP535336.1 | MPXV_Nigeria_2017_2946 |
| MT903339.1 | MPXV-M3021_Delta | NC_063383.1 | Monkeypox virus |
| MT903337.1 | MPXV-M2940_FCT | OP535329.1 | MPXV_Nigeria_2018_5305 |
| **Monkeypox virus Clade IIb Lineage A.1** | | | |
| MT903343.1 | MPXV-UK_P1 | MT903345.1 | MPXV-UK_P3 |
| MT903342.1 | MPXV-Singapore | MN648051.1 | Israel_2018 |
| OP612686.1 | MPXV/053/19 | MT250197.1 | Singapore 2019 |
| OP612682.1 | MPXV/040/19 | | |
| **Monkeypox virus Clade IIb Lineage A.1.1.1** | | | |
| ON676708.1 | MPXV_USA_2021_MD | | |
| **Monkeypox virus Clade IIb Lineage A.2** | | | |
| OP642362.1 | MCL-22-H-MPxV-16-5316 | ON675438.1 | ON675438.1 |
| OP612691.1 | OP612691.1 | PP853037.1 | PP853037.1 |
| **Monkeypox virus Clade IIb Lineage A.2.1** | | | |
| PQ178860.1 | Monkeypox/PT0824/2024 | OP605572.1 | Slovenia-MPXV-30-2022 |

| | | | |
|---|---|---|---|
| PQ059889.1 | hMpxV/Austria/MUW1721722/2024 | ON674051.1 | MPXV_USA_2022_FL001 |
| OR459777.1 | MPXV-ROK-P004-2022 | | |
| **Monkeypox virus Clade IIb Lineage A.2.2** | | | |
| PQ159993.1 | hMpxV/USA/CA-CDPH-1M1000642/2024 | PP852977.1 | TRM082 |
| PP852972.1 | VSP215 | OR113690.1 | hMPXV/Austria/MUW1607960/2023 |
| **Monkeypox virus Clade IIb Lineage A.2.3** | | | |
| PP852951.1 | VSP193 | OP555515.1 | Monkeypox/PT0428/2022 |
| PP853035.1 | TRM341 | | |
| **Monkeypox virus Clade IIb Lineage A.3** | | | |
| PP852953.1 | VSP195 | OP535335.1 | MPXV_Nigeria_2017_2947 |
| OP413718.1 | 9000360 | OP536812.1 | 9000450 |
| **Monkeypox virus Clade IIb Lineage B.1** | | | |
| OP390199.1 | hMpxV/UK/LMO-034a/2022 | OQ627926.1 | INMI-22932022 |
| OP324541.1 | Monkeypox/PT0233/2022 | OR146397.1 | Z22IRL00522 |
| OR035649.1 | MPXV/UZ_REGA_43 | OP160532.1 | hMpxV/Netherlands/NH-AUMC-0001/2022 |
| OP536760.1 | 9000381 | ON751962.1 | Monkeypox/BR0001/2022 |
| ON649879.1 | MPXV_ISR001_2022 | ON782022.1 | MPX-42/Finland/2022 |
| OP422341.1 | hMPXV/Austria/MUW1538395/2022 | | |
| **Monkeypox virus Clade IIb Lineage B.1.1** | | | |
| OP743955.1 | MpxV/human/USA/WA-UW-089756/2022 | ON929064.1 | Monkeypox/PT0564/2022 |
| OP484684.1 | MPXV/Germany/2022/RKI363 | OP484669.1 | MPXV/Germany/2022/RKI348 |
| OP555635.1 | Monkeypox/PT0564/2022 | | |
| **Monkeypox virus Clade IIb Lineage B.1.2** | | | |
| PP098591.2 | MPXV/B4 | OR499953.1 | MPXV/Human/USA/CA-LACPHL-MA00496/2023 |
| OR427343.1 | MpxV/human/USA/WA-UW-038917/2023 | OR427337.1 | MpxV/human/USA/WA-UW-013706/2023 |
| **Monkeypox virus Clade IIb Lineage B.1.3** | | | |
| PP057709.1 | MPXV/AR-ANLIS008/2022 | OP605554.1 | Slovenia-MPXV-11-2022 |
| PP057709.1 | MPXV/AR-ANLIS008/2022 | ON983168.1 | MPxV/Prague_001/2022/flye_medaka |
| **Monkeypox virus Clade IIb Lineage B.1.4** | | | |
| OR759156.1 | hMpxV/Canada/ON-PHOL066/2022 | OP279037.1 | MpxV/human/CAN/UN-NML-4113/2022 |
| OR126132.1 | HRYC_MPXV002 | | |
| **Monkeypox virus Clade IIb Lineage B.1.5** | | | |
| ON918656.1 | MPXV/human/Taiwan/110-231642/2022 | OP783902.1 | hMPXV-CH-38347314/2022 |
| **Monkeypox virus Clade IIb Lineage B.1.6** | | | |
| OP320553.1 | LIM-INS-013 | OP289783.1 | LIM-INS-003 |
| **Monkeypox virus Clade IIb Lineage B.1.7** | | | |
| ON911481.2 | Monkeypox/MX-UANL001/2022 | ON927248.1 | NICD-SVPL232 |
| OR146401.1 | Z22IRL00482 | ON918611.1 | NICD-SVPL223 |
| **Monkeypox virus Clade IIb Lineage B.1.8** | | | |
| OQ320513.1 | MPXV_USA_2022_FL0009 | OR353588.1 | MN-MDH-MPXV_0078 |
| **Monkeypox virus Clade IIb Lineage B.1.9** | | | |
| OP555631.1 | Monkeypox/PT0560/2022 | OP555608.1 | Monkeypox/PT0537/2022 |
| OR146285.1 | Z22IRL00518 | | |
| **Monkeypox virus Clade IIb Lineage B.1.10** | | | |
| ON959143.1 | MPX-96/Finland/2022 | OP450985.1 | MPXV_USA_2022_TX0019 |
| **Monkeypox virus Clade IIb Lineage B.1.11** | | | |
| OR464206.1 | MPXV_USA_2022_TN0020 | OR427333.1 | MpxV/human/USA/WA-UW-129402/2022 |
| **Monkeypox virus Clade IIb Lineage B.1.12** | | | |
| ON880548.1 | MpxV/human/CAN/UN-NML-3448/2022 | OQ918701.1 | MPXV/Human/USA/CA-LACPHL-MA00490/2023 |
| **Monkeypox virus Clade IIb Lineage B.1.13** | | | |
| OR160418.1 | hMpxV/USA/IL-RIPHL-MPXV-052-0034/2023 | OP743957.1 | MpxV/human/USA/OH-UW-080634/2022 |
| **Monkeypox virus Clade IIb Lineage B.1.14** | | | |
| OP555644.1 | Monkeypox/PT0573/2022 | OP555562.1 | Monkeypox/PT0486/2022 |
| OP382493.1 | hMPXV-IHU00016 | OX009124.1 | Monkeypox virus isolate Lesion genome assembly, chromosome: 1 |
| **Monkeypox virus Clade IIb Lineage B.1.15** | | | |
| OP819950.1 | MET_INS_MPX_0956_C | OP484673.1 | MPXV/Germany/2022/RKI35 |
| **Monkeypox virus Clade IIb Lineage B.1.16** | | | |

| | | | |
|---|---|---|---|
| OP390195.1 | hMpxV/UK/LMO-029a/2022 | OP819949.1 | VAC_INS_MPX_0955_C |
| **Monkeypox virus Clade IIb Lineage B.1.17** | | | |
| OQ991943.1 | strain B.1.17 | OQ547883.2 | hMpxV/USA/CACDPH1MPX1000175/2022 |
| **Monkeypox virus Clade IIb Lineage B.1.18** | | | |
| OQ503770.1 | MpxV/human/USA/WA-UW-096758/2022 | OQ330978.1 | Monkeypox virus/Human/USA/CA-LACPHL-MA00375/2022 |
| **Monkeypox virus Clade IIb Lineage B.1.19** | | | |
| OP555643.1 | Monkeypox/PT0572/2022 | OP555661.1 | Monkeypox/PT0594/2022 |
| **Monkeypox virus Clade IIb Lineage B.1.20** | | | |
| PQ328533.1 | WA-UW-032044 | PQ328530.1 | WA-UW-425252 |
| **Monkeypox virus Clade IIb Lineage B.1.21** | | | |
| PQ159966.1 | hMpxV/USA/CA-CDPH-1M1000608/2024 | PQ159965.1 | hMpxV/USA/CA-CDPH-1M1000607/2024 |
| **Monkeypox virus Clade IIb Lineage B.1.22** | | | |
| PQ155106.1 | Monkeypox virus/Human/USA/CA-LACPHL-MA00651/2024 | PQ274319.1 | hMpxV/USA/CA-CDPH-1M1000646/2024 |
| **Monkeypox virus Clade IIb Lineage C.1** | | | |
| LC831698.1 | MPXV/human/Japan/Tokyo/NCGM240303/2024 | OQ721954.1 | MPXV/human/Taiwan/110-539498/2023 |
| OR855755.1 | MPXV-ROK-P53/12-2023 | LC753969.1 | MPXV/human/Japan/Tokyo/2022/TKY220176 |
| PQ153226.1 | hMpxV/THA/V241-0052/2023 | Thailand | |
| **Monkeypox virus Clade IIb Lineage C.1.1** | | | |
| PP648206.1 | MPXV/human/CHN/GDCDC_SZ_M23022/2023 | PP882804.1 | MPXV_USA_2024_MA0223 |
| OR601604.1 | Z22IRL00908 | PP481193.1 | MPXV/PT0814/2024 |
| OR804493.1 | MPV/PT0725/2023 | | |

**Cross-reactivity - Analytical Specificity**

Wet testing analysis was performed against the following respiratory pathogens. No cross-reactivity was observed with any pathogen mentioned below.

| | |
|---|---|
| *Staphylococcus aureus* | *Pseudomonas aeruginosa* |
| *Escherichia coli* | *Klebsiella pneumoniae* |
| *Enterococcus faecalis* | *Staphylococcus epidermidis* |

In addition, a separate *in-silico* analysis was performed for the following pathogens not available for wet testing. The Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit shows 100% specificity to monkeypox virus only.

| | |
|---|---|
| *Streptococcus pyogenes* | Camelpox virus |
| *Diphtheroid* | Cowpox |
| *Peptostreptococcus anaerobius* | Vaccinia |
| *Propionibacterium acnes* | Variola |
| *Streptococcus bovis* | Taterapox |
| Raccoonpox | Ectromelia |
| Skunkpox | Herpesvirus |
| Leporipox | Rickettsia |

**Warning**

Not for Medicinal Use.

**Precautions and Limitations**

Strict compliance with the Instructions for Use is required for optimal results and the use of the kit is limited to staff qualified clinical laboratory personnel trained in the techniques of real-time PCR and in vitro diagnostic procedures. Good clinical laboratory practices are to be followed while handling clinical samples with appropriate PPEs.

Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. This assay must not be used on the specimen directly. Viral DNA should be extracted from human serum samples using appropriate nucleic acid extraction method. Presence of PCR inhibitors and other interferences may lead to false negative or invalid results. Although rare, mutations within the highly conserved regions of the targets genes covered by the kit's primers and/or probe may result in failure to detect the presence of pathogen. As with any diagnostic test, results of the Hi-PCR® Monkeypox Virus Multiplex Probe PCR need to be interpreted in consideration of all clinical and laboratory findings. Performance of the kit in monitoring treatment of Monkeypox infection has not been evaluated.

**Quality Control**

The Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit provides controls- Positive Control and a Negative Template Control (NTC) which are to be included in every run.

**Evaluation**

Every lot of Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit would be tested against predetermined specifications to ensure consistent product quality.

**Troubleshooting Guide**

| Sr. No. | Problem | Cause | Solution |
|---|---|---|---|
| 1. | No amplification | Degraded samples | Check the integrity of nucleic acid using agarose gel electrophoresis. |
| | | | Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification. |
| | | Error in protocol setup | Check whether all components are added in correct volume as per the manual. |
| | | Inappropriate storage conditions | Store the reagents at recommended temperature for its optimal performance. Check expiry of the reagents and use new lot of reagents if necessary. |
| | | Incorrect PCR programming | Ensure selection of appropriate fluorescence channel as detailed in the manual.<br>Compare the PCR program to the manual. |
| 2. | Variability between replicates | Error in reaction set-up | Prepare a large volume master mix, vortex thoroughly and aliquot into reaction tubes. |
| | | Air bubbles in reaction mix | Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument. |
| | | Pipetting error | $C_t$ values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes. Use calibrated pipettes. Repeat the run. |
| 3. | Amplification in No template control | Cross contamination during handling | Replace all critical solutions.<br>Repeat the analysis of all tests with fresh aliquots of critical reagents.<br>Follow good laboratory practices to avoid contamination issues. |

**Safety Information**

HiMedia's Hi-PCR® Monkeypox Virus Multiplex Probe PCR Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

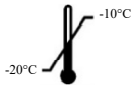
**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

**Technical Assistance**

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

**Symbols**

| | | | |
|---|---|---|---|
|  | Manufacturer |  | Do not use if package is damaged |
|  | Batch code |  | Temperature limit |
|  | Date of manufacture (YYYY-MM) |  | Consult instructions for use |
|  | Use-by date (YYYY-MM) |  | Catalogue number |

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