

AstraArt Monkey Pox qPCR Kit (Generic)

PRINCIPLE

AstraArt Monkey Pox qPCR Kit (Generic) is a real-time polymerase chain reaction assay for the qualitative detection of Monkey Pox virus (MPox) gene target in samples collected from the individuals suspected of Monkey Pox infection. The assay is a combination of the latest advanced buffer chemistry, PCR enhancers and stabilizers along with antibody-mediated hot-start polymerase, dNTPs and MgCl₂. This assay has been designed for highly reproducible, accurate results in the presence of inhibitors, making it ideal for detecting lowest copy numbers of MPox target genes.

The MPox primer and probe set(s) are designed to detect specific target sequence of TNF receptor gene to identify Monkey pox virus and the Internal Control (IC) gene.

PACKAGE CONTENTS

Description	Specification	Quantity for 100 tests
qPCR Master Mix	qPCR amplification Mix	1000 μ l x 1 tube
Primer mix	MPox Primer Probe mix	500 μ l x 1 tube
Positive Control	DNA Positive Control	100 μ l x 1 tube
Negative control	No Template Control	100 μ l x 1 tube
Catalogue Number	AG/MPX/22/02	

STORAGE & STABILITY

- All the reagents should be stored at -20 °C. Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. Avoid repeated freeze/thaw cycles for reagents.

SAMPLE REQUIREMENTS

- Specimens collected for MPX 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
- It is ideal to carry out extraction protocol with fresh samples.
- Repeated freezing and thawing should be avoided.
- Transport the specimens at a temperature between 2 to 8°C.

SAMPLE COLLECTION

- Swabs from skin lesion including lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts can be collected. In case of contacts who have been exposed to MPox, a swab from nasopharyngeal or oropharyngeal surface can be collected for initial screening.

ASSAY PROCEDURE:

Nucleic acid isolated from the sample using any validated DNA extraction system. The extracted DNA is directly amplified using the AstraArt Monkey Pox qPCR Kit (Generic) on the Real-time PCR Instrument system. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Real-time PCR Instrument system.

- Preparation of Real-time PCR reagents: Briefly Centrifuge all the reagents. Prepare the reagents according to the table below. The volume of each reagent is calculated by multiplying the number of samples by the volume of each component in Table 1 accordingly.

Table 1: Components of Master mix:

Components	Volume (μ l) per reaction
qPCR Master Mix	10
Primer Mix	5
Total volume	15μl

- Mix the reaction master mix and spin-down briefly. Aliquot 15 μ l of the master mix into each well of 96-well plate and add 5 μ l of the sample DNA/Negative control/Positive control accordingly. Seal the plate and spin-down briefly. Run the Protocol immediately on the Real-time PCR instrument with following cycling conditions in Table 2.

Table 2: Cycling Conditions:

Steps	Temperature °C	Time	Cycle
1	95	3 minutes	1
2	95	10 seconds	35
3	60*	30 seconds	
	MPox uses the HEX (or VIC) channel, and the IC uses the CY5 channel. *Fluorescence is measured at 60° C.		

Note: Please select "None" in both Passive reference and Quencher.

c. Interpretation of Results:

Interpret the values for unknown samples based on the observations as described in the following table. ≤ 32 Ct of unknown samples should be considered for result interpretation. The lower limit of detection (LOD) of AstraArt Monkey Pox qPCR Kit (Generic) is defined as 16 copies/reaction.

Table 3: Determination of CT value:

Fluorescent Channel	CT value	Result
MPox (HEX/VIC)	≤ 32	+
IC (Cy5)	≤ 32	+

Table 4: Conclusion:

	MP0x (HEX/VIC)	IC (Cy5)	Conclusion
Negative Control	-	-	Valid
Positive control	+	-	Valid
Sample	+	+	Positive for MPox
Sample	+	-	Positive for MPox
Sample	-	-	Invalid run repeat test with fresh sample
Sample	-	+	Negative for MPox

LIMITATION OF THE PROCEDURE:

1. This kit is used for qualitative detection of Monkey Pox DNA from specimens. The results do not reflect the viral load in the original specimen.
2. The specimen to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
3. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure.

WARNING & PRECAUTIONS:

- Do not use the product if there is evidence of leakage.
- Adhere to standard procedures and published protocols for sample collection, processing, and disposal.

SYMBOLS:



Do not use if package is damaged



CE mark of Conformity



Refer to the instructions



ISO



GMP



AstraGene FZ LLC

Office No. 208 – 209, Dubai Science Park Building, Dubai, United Arab Emirates

+971-4-8781222, contact@astragene.com