AMA Monkeypox Multiplex qPCR Detection Kit

Product Manual

Version 1.0

This product is intended for research use only.

Contents

A. Introduction and Principles of the Test

This fourplex, probe-based assay is intended for detection of the Monkeypox virus in human clinical samples. Two highly conserved Monkeypox virus-specific genomic regions are targeted by this assay; the first spans the C14L gene and its flanking region, while the second is an intergenic region. This assay also targets a region on the DNA polymerase gene that is highly conserved in all orthopox viruses, which enables laboratories interested in Monkeypox detection to also be able to monitor infections caused by other orthopox viruses.

Target	Monkeypox1 (Intergenic)	Monkeypox2 (C14L)	Orthopox (DNA Pol)	IC (Human Chr6)
Detection Channel	FAM	HEX	Cy5	ROX

The fourth target in this assay is a DNA segment on the human chromosome6, which severs as the internal control of the assay. Although synthetic spike-ins are sufficient controls for the extraction, amplification, and detection steps, they do not provide enough information about the state of the original sample during the collection and transportation steps. This could be problematic for degraded samples, which may be incorrectly called negative. By targeting human DNA, this assay overcomes this problem while providing all of the necessary controls that spike-ins provide.

As of June1, 2022, there were 417 complete sequences published on the NCBI Virus database, of which 100 were Monkeypox sequences. The oligos that target orthopox viruses in this assay have 99.3% perfect complementarity to all of these sequences. On the other hand, the two Monkeypox-specific oligos have 100% complementarity to all Monkeypox sequences, while not complementary to non-Monkeypox orthopox viruses.

In-silico analysis shows that our assay has no cross-reactivity with any other viruses or bacteria.

B. Kit Contents and Storage

The kit comes in a package of four tubes, as indicated in the following table. All components must be stored at -20°C. The qPCR Master Mix and Oligo Mix should not be exposed to light for extended periods of time.

Component	Volume (uL)
qPCR Master Mix	1,000
AMA Oligo Mix	200
Negative Control	50
Positive Control	50

C. Protocol

1. Extract DNA or total nucleic acids from each clinical sample, as in your laboratory's approved internal protocol. Nucleic acid should be used immediately after extraction (within 30 minutes) or stored at -20°C immediately after extraction.

2. Thaw all components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. ***Do NOT exceed 3 freeze-thaw cycles!***

3. Prepare a reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):

    a. *10 µL of qPCR Master Mix / reaction*
    b. *2 µL of the AMA Oligo Mix / reaction*

Example

Component	Per 1 reaction	X10 reactions (+5% overage)
qPCR Master Mix	*10 µL*	*105 µL*
AMA Oligo Mix	*2 µL*	*21 µL*

NOTE: Don't forget to figure in the positive and negative controls in your calculations!

4. Transfer 12 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

6. Seal the plate with optical cap strips or an optical adhesive seal.

7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	Monkeypox1	Monkeypox2	Orthopox	IC
Detection Channel	FAM	HEX	Cy5	ROX

8. On the software, define your Positive and Negative Controls.

9. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

Step1:	*3 minutes - 95°C (initial denaturation)*
Step2:	*15 seconds - 95°C (denaturation)*
Step3:	*60 seconds - 60°C (annealing/extension)* → ***DATA COLLECTION***
Step4:	*go to step2 (X39 times)*

10. Analyze the results according to your thermal cycler's software.

D. Data Interpretation

Sample calling

Our assay merely provides Ct values, and interpretation of these values should be in accordance with the international standards. Here, we summarize our recommendations for sample calling in the following table, whereas the + sign indicates detection (Ct < 40) and the – sign indicates no detection (Ct ≥ 40):

It is important to note that acceptable cycle threshold of detection is Ct ≤ 40, which means that Ct > 40 should be neglected.

	ORTHO (CY5)	MONKPX1 (FAM)	MONKPX2 (HEX)	IC (ROX)	INTERPRETATION
CASE1	+	+	+	+ or –	**Monkeypox infection**
CASE 2	–	+	+	+ or –	**Monkeypox infection**
CASE 3	+	–	+	+ or –	**Monkeypox infection**
CASE 4	+	+	–	+ or –	**Monkeypox infection**
CASE 5	–	–	+	+	**Monkeypox infection**
CASE 6	–	+	–	+	**Monkeypox infection**
CASE 7	+	–	–	+	**Orthopox infection**
CASE 8	–	–	+	–	**Presumptive Monkeypox , retest**
CASE 9	–	+	–	–	**Presumptive Monkeypox , retest**
CASE 10	+	–	–	–	**Presumptive Orthopox , retest**
CASE 11	–	–	–	+	**No infection**
CASE 12	–	–	–	–	**Sample degraded**

Run validity

This assay contains positive and negative controls. The three targets and internal control must all be detected in the positive control; if not, the whole plate is considered invalid. Similarly, the negative control must show detection of the internal control alone; any other result invalidates the whole plate.

E. Support

For questions, suggestions, or technical support, feel free to contact us by email on:

support@havensci.com

F. Quality Control

Each lot is tested against predetermined parameters to ensure consistent performance among all lots.