RayBio® Monkeypox Virus (MPXV) 1-Step High Throughput PCR Kit

Catalog #: PCR-MPXV-HTOS

User Manual
Last revised: 09-23-2022

Caution:
Extraordinarily useful information enclosed

ISO 13485:2016

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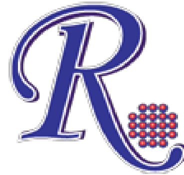

RayBiotech, Inc.

RayBio® Monkeypox Virus (MPXV) 1-Step High Throughput PCR Kit Protocol

Please read the entire manual carefully before starting your experiment

INTRODUCTION

Monkeypox virus is a virus from the orthopox family of viruses, a family most often associated with the more severe smallpox virus (variola), or the origination of vaccinations from the cowpox virus. Originally named for an outbreak among monkeys, it was first identified in humans in central and western Africa around 1970 where it continues to circulate at a low endemic level. While the virus is clinically less severe than smallpox, the symptoms are similar. With vaccinations against smallpox ceasing in 1972 and the eradication of the smallpox virus, vaccinations in the younger population (<50 years of age) is extremely limited. The escape from the endemic regions has the World Health Organization on alert given the lack of associated travel within the region of those suspected and confirmed cases. Understanding how the virus is being spread, the populations it is spreading to, and the potential immune responses against the virus are of increasing importance in the research field.

The RayBiotech Monkeypox Virus 1-Step High Throughput PCR Kit is a ready to use PCR assay for the detection of MPXV DNA. It uses a fluorescent probe with specific primer sets to detect the J2R gene specific for the genome of MPXV. Primer and probe for an internal control, RNase P are also integrated in the assay to validate the assay quality. The kit also contains a proprietary buffer which enables the user to use crude skin swabs directly in their PCR reactions. By simply mixing the 1-Step Nucleic Acid Stabilization Buffer with the sample, the viral DNA is immediately released and stabilized, and interfering components of the human tissue are inactivated. This eliminates the need for laborious, costly DNA extraction and purification steps, drastically improving upon the traditional workflow of PCR, while removing potential sample loss and contamination.

PACKAGING SPECIFICATIONS

96 tests/box

PURPOSE

This kit is used for the qualitative *in vitro* detection of *Monkeypox virus* (MPXV) nucleic acid from skin swap samples in VTM mixed with 1-Step Nucleic Acid Stabilization Buffer.

KIT COMPONENTS

Component	Catalogue #	Ingredients	Specification	Quantity
2x PCR Reaction Solution	PCR-MPXV-MM	Buffer, dNTP's, enzyme, ROX reference dye	1000µL / tube	1 tube
Primers and Probe Mix	PCR-MPXV-PP	MPXV/RNase P Primers & Probe Mix	500µL / tube	1 tube
Positive Control	PCR-POS-POS	Synthetic MPXV genomic DNA/RNase P Positive Control	50µL / tube	1 tube
Negative Control	PCR-MPXV-NEG	Nuclease-free water	500µL / tube	1 tube
1-Step Nucleic Acid Stabilization Buffer		Buffer	10mL / bottle	1 bottle

Note: Do not mix reagents from different lots.
Note: PCR machines may require specific PCR plate types. Please refer to the manufacturer's recommendation for PCR plates before running the assay on your PCR machine.

STORAGE AND EXPIRATION
The kit can be stored at -20°C for a period of 12 months prior to opening. After opening, the reagents are valid for at least 6 months if stored at -20°C.

REQUIRED MATERIALS (NOT INCLUDED)
- VTN DNA preservation solution
- Fluorescence PCR instrument capable of reading FAM or equivalent channel (494 nm maximum absorption, 518 nm maximum emission) and TAMRA or equivalent channel (550 nm maximum absorption, 518 nm maximum emission).
- Vortex Mixer
- Microcentrifuge
- Pipettes
- Sterile nuclease-free pipette tips (barrier tips recommended) and microfuge tubes
- Compatible PCR Plate
- Contact our technical support team for questions about compatibility: techsupport@raybiotech.com

SAMPLE REQUIREMENTS
1. Sample type: skin swabs stored in VTM (viral transport medium).
2. Samples should be regarded as a potential source of infection. Sample handling should be performed in a microbiological and biomedical laboratory with an appropriate biosafety label to protect the operator from possible exposure during work.
3. All specimens, regardless of how or for what purpose they were collected, should be regarded as potentially infectious and handled with extreme caution. Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing any samples. These precautions, at a minimum, should include proficiency and competency testing, appropriate PPE, avoiding aerosols, and using effective disinfectants (quaternary ammonium compounds and 0.5% bleach).

GENERAL CONSIDERATIONS
1. To prevent contamination of PCR reactions, clean and decontaminate all working surfaces, centrifuges, pipets, and other equipment with 10% bleach or DNA Away®, followed by 70% Ethanol before every assay.
2. Conduct sample processing and DNA extraction in a separate area (below termed the "**Sample Processing Area**") from the PCR assay setup (below termed the "**PCR Assay Setup Area**").

3. Care should be taken to avoid contamination of samples and reactions with DNA amplicons from used PCR plates. It is thus recommended to place used PCR plates in a sealed bag and discard them appropriately. Never open a used PCR plate or place it in the same area as the PCR instrument.

4. To minimize cross-contamination between experimental samples, disposable pipettes and filtered pipette tips are recommended.

TESTING METHOD

1. Sample Processing (Sample Processing Area)

1.1. **Sample Inactivation and Preservation**: Use a VTM DNA sample preservation solution for virus inactivation and DNA preservation.

1.2. **For Swabs**: For swabs, vortex or mix the swab/VTM tube briefly to ensure the VTM is well mixed. In a sterile microfuge tube, mix 100 µL of the 1-Step Nucleic Acid Stabilization Buffer with 100 µL of the VTM the swab is stored in. Cap and vortex for 30 seconds. **This solution will be used in Step 3.2**. NOTE: Before storing the remaining solution in the tube with the swab, briefly centrifuge the tube to collect the solution at the bottom of the tube. A precipitate may be present at the bottom of the tube. Store at 4ºC for up to 24 hours before use or freeze for long term storage.

2. Assay Assembly (PCR Assay Setup Area)

2.1. **Thaw reagents:** Remove all components from the kit, and fully thaw to room temperature. After thawing, mix gently by pipetting. Briefly centrifuge to collect contents at bottom of vial.

2.2. **Calculate number of reactions needed:** The number of reactions to be prepared per PCR run maybe calculated by (# of singly run samples to be tested + 2). Adding 2 to the number of samples to be tested takes positive and negative controls tests into account. It is recommended to include 1 positive and 1 negative control with each experiment. Refer to Table 1 for a summary of reaction components included in each well. NOTE: it is recommended to make 1 or 2 additional reaction volumes to account for pipetting error.

2.3. **Prepare PCR Master Mix:** As outlined in Table 1, each reaction should contain 10µL 2x PCR Reaction Solution and 5 µL Primers and Probe Mix. To calculate the total volume necessary for the run, multiply the volumes of each component by the number of reactions calculated in 2.2 above. Mix the PCR Reaction Solution and other components together to prepare a Master Mix.

Table 1: Reaction Components for Samples and Controls.

Component	Positive Control Reaction	Negative Control Reaction	Sample Reaction
2xPCR Reaction Solution	10 µl	10 µl	10 µl
Primers and Probe Mix	5 µl	5 µl	5 µl
Positive Control	5 µl	--	--
Negative Control	--	5 µl	--
Sample	--	--	5 µl
Total Volume	**20µl**	**20µl**	**20µl**

3. Sample Loading (PCR Assay Setup Area)

3.1 Add 15 µl of prepared PCR Master Mix from 2.3 above to each well of a PCR reaction plate.

3.2 Add 5 µl of sample template (prepared Step 1) to each well and pipette up and down at least 5 times to mix.

3.3 Add at least 1 positive control and 1 negative control samples.

3.4 Seal the plate or tubes tightly.

3.5 Centrifuge the plate or tubes for 30 seconds at low speed. Note: The sealed PCR reaction tubes can be stored at 2-8°C for up to 4 hours before the “PCR Amplification” step 4 below.

4. PCR Amplification (PCR Assay Setup Area)

4.1 **Sample setup:** Set the sample number, targets, negative control, and positive control accordingly to your

plate setup.

4.2 **Fluorescence Channel Selection:** Select FAM (or equivalent channel) and set the target name for “MPXV”, this channel will detect the J2R viral gene. Select TAMRA (or equivalent channel) and set the target name for “Internal Control”, this channel will detect the RNase P gene. This kit contains a reference fluorescence dye (passive reference) that will help decrease variation. In the PCR instrument setting, set the reference dye to “ROX”.

4.3 Set reaction conditions according to Table 2. Set the reaction volume to 20 µl.

Table 2: PCR Program

Step		Temperature (°C)	Time	Temperature Ramp Rate	Number of Cycles
Stage 1	Initial Denaturation	95	1 min	1.6°C/sec	1
Stage 2	Denature	95	15 s	1.6°C/sec	40
	Anneal, extend, detect fluorescence	60	30 s	1.6°C/sec	

4.4 Save the file and run program. A sample image of PCR amplification of serially diluted MonkeyPox Synthetic Genomic DNA is shown (Figure1).

5. Result and Analysis

The positive and negative control PCR reactions are considered valid if the negative and positive controls meet the criteria listed in Table 3. The PCR reaction is invalid if 1) the positive control does not have logarithmic growth or the Ct ≥ 36 or 2) the negative control has a Ct < 36. If the reaction is invalid, the measurement of all samples in this experiment should be discarded, and the assay repeated.

Note: Since RNase P signal on TAMRA channel has lower background than Monkeypox signal on FAM channel, researchers can present the two targets separately.

Table 3. **Validation of PCR reactions with quality controls**

Target	Positive Control	Negative Control
MPXV	Ct < 36	Ct ≥ 36 or no Amplification
RNase P	Ct < 36	Ct ≥ 36 or no Amplification

INTERPRETATION OF TEST RESULTS

The PCR reaction results are explained according to Tables 4 and 5.

Table 4. **Interpretation of Individual PCR Reactions**

PCR reaction results	MPXV Ct	RNase P
+	< 36	< 36
-	No amplification, or Ct ≥ 36	No amplification, or Ct ≥ 36

Table 5. **Interpretation of Sample Test**

FAM (MPXV detection)	TAMRA (PCR validation)	PCR Result
+	+	MPXV +
+	-	MPXV +
-	+	MPXV -
-	-	Invalid PCR

Positive Result: the sample contains the target genes.

Negative Result: the sample does not contain the target genes.

Invalid Result: the sample should be rerun with fresh samples and controls.

PRODUCT PERFORMANCE INDEX

1. **Limit of Detection:** The LOD of the assay is 4 copies/μL, or 20 copies per reaction (5μL of sample volume).
2. **Repeatability:** Precision testing showed that the coefficient of variation of the precision Ct values within this kit lot are ≤ 1.2%. Repeatability between lots of product are to be ≤ 10%.

Specificity

No cross reactivity was identified when the kit was evaluated against other common Orthopox family members: Cowpox virus, Camelpox virus, and Vaccinia virus.

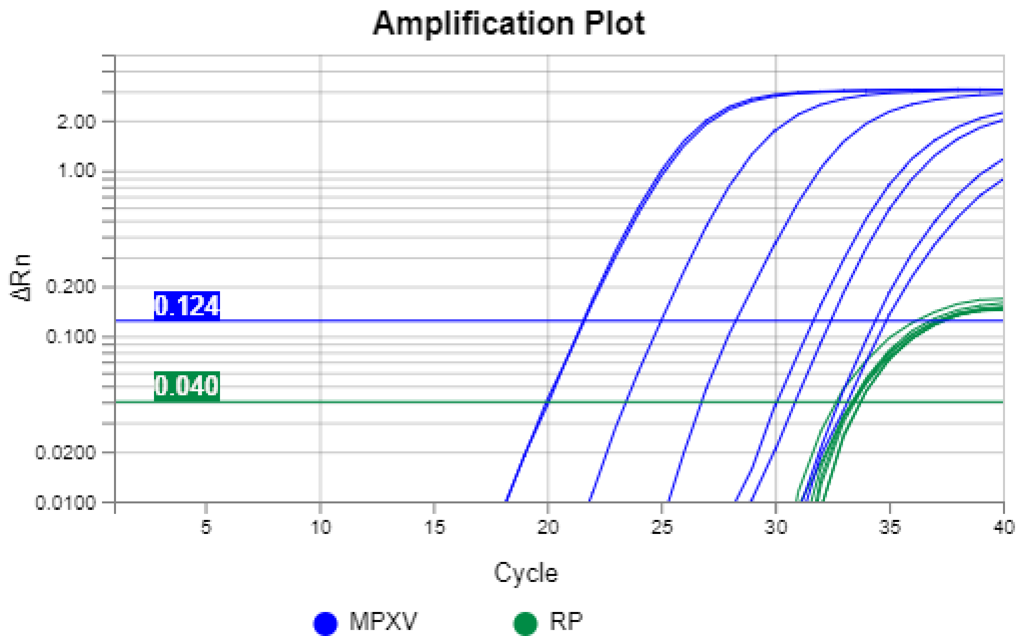

Figure 1. Serially diluted Monkeypox Virus synthetic genomic DNA and fixed amount of Human RNase P DNA was spiked in VTM solution and mixed with equal volume of 1-Step Nucleic Acid Stabilization Buffer. Five microliter of the DNA-spiked sample that contains 10^4, 10^3, 10^2, 10, 5, 2.5 and 1.25 copies/μl of Monkeypox Virus Synthetic Genomic DNA (MPXV, blue color) and fixed amount of RNase P template (RP, green color) were added to 15ul of master mix and amplified in a QuantStudio™ 5 Real-Time PCR.