RayBio® Mpox Virus (MPXV) PCR Nucleic Acid Detection Kit

Catalog #: PCR-MPXV

User Manual
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Caution:
Extraordinarily useful information enclosed

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RayBio® Mpox Virus (MPXV) PCR Nucleic Acid Detection Kit Protocol

Please read the entire manual carefully before starting your experiment

INTRODUCTION

Mpox 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 Mpox Virus PCR Nucleic Acid Detection Kit is a ready to use PCR assay for the detection of MPXV DNA in a liquid sample. It uses two sets of specific primers and probes at the same fluorescent channel to detect the J2L and B6R genes of MPXV to enhance its sensitivity. A set of primers specific to a conserved region of all orthopox viruses were also used to evaluate possible false negative caused by virus genome mutation. Primer and probe for an internal control, RNase P are also integrated in the assay to validate the assay quality.

PACKAGING SPECIFICATIONS

96 tests/box

PURPOSE

This kit is used for the qualitative *in vitro* detection of *Mpox virus* (MPXV) nucleic acid from purified DNA samples. Sample DNA should be purified according to manufacturer or related procedures.

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	MPXVJ2L, B6R/Orthopox E9L/RNase P Primer & 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

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)

- Sample DNA purification: can be any common commercially available genomic DNA purifications kits
- DNA preservation solution
- Fluorescence PCR instrument capable of reading FAM or equivalent channel (494 nm maximum absorption, 518 nm maximum emission), Cy5 channel (640 nm maximum absorption, 682 nm maximum emission) and TAMRA or equivalent channel (550 nm maximum absorption, 586 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. This is a Research Use Only (RUO) kit and is not to be used for diagnostic purposes of any kind.
2. Sample types: Samples should be purified DNA using commonly available lab practices like

Trizol related methods, or commercially available DNA purification kits. Please follow manufacturer's guidelines with respect to any sample purification steps. The final sample DNA amount added to the assay should not exceed 100 ng.

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 DNA sample preservation solution for virus inactivation and DNA preservation.

   1.2. **Sample Extraction and Purification**: Common commercial nucleic acid extraction and purification kits, or Trizol-based extraction method, may be used to extract the nucleic acid.

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 DNA (no more than 100ng recommended) 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 both J2L and B6R viral gene. Select TAMRA (or equivalent channel) and set the target name for “Internal Control”, this channel will detect the RNase P gene. Select Cy5 (or equivalent channel) and set the target name for “Orthopox” E9L gene. This kit contains a reference fluorescence dye ROX (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 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 Mpox signal on FAM channel and Orthopox signal on Cy5 channel, researchers can present the RNase P separately.

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

Target	Positive Control	Negative Control
MPXV B6R/J2L	Ct < 36	Ct ≥ 36 or no Amplification
Orthopox E9L	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)	Cy5 (Orthopox virus detection)	TAMRA (PCR validation)	PCR Result
+	+	+	MPXV +
+	+	-	MPXV +
-	-	+	MPXV -
-	+	+	Retest needed
-	-	-	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 2 copies/µL, or 10 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.

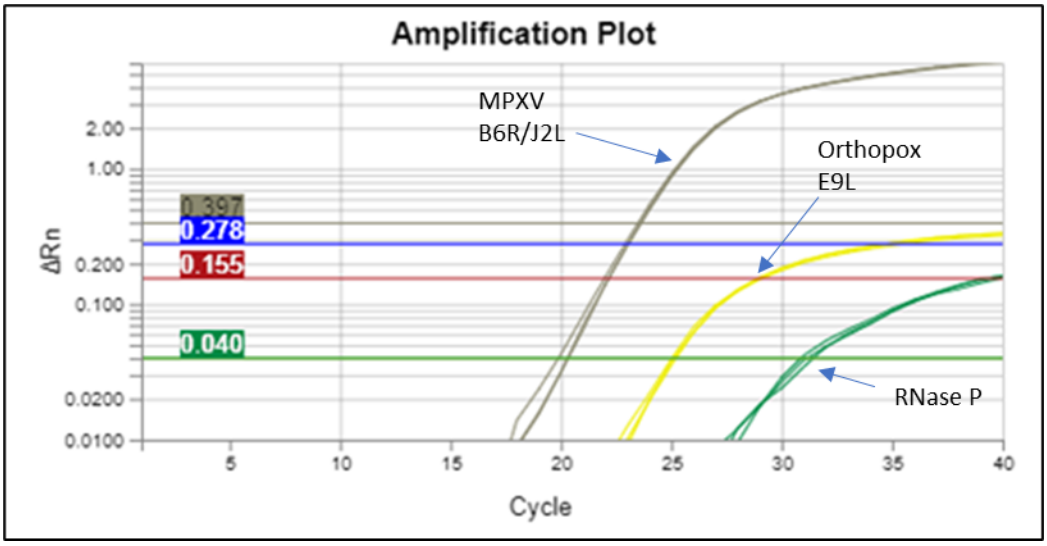

Figure 1. Five microliter of positive control contains 10^2 copies/µl of Mpox Virus B6R and J2L Synthetic DNA (Grey color, FAM channel), 10^2 copies/µl of Orthopox virus J2L Synthetic DNA (Yellow color, Cy5 channel) and fixed amount of RNase P template (Green color, TAMRA channel) were added to 15ul of master mix and amplified in a QuantStudio™ 5 Real-Time PCR.