# RayBio® Mpox Virus (MPXV) Clade I & II PCR Nucleic Acid Detection Kit

Catalog #: PCR-MPXV-CLADE

User Manual
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Caution:
Extraordinarily useful information enclosed

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3607 Parkway Lane, Suite 100
Peachtree Corners, GA 30092
Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax:770-206-2393
Web: www.RayBiotech.com, Email: info@raybiotech.com

RayBiotech, Inc.

# RayBio® Mpox Virus (MPXV) Clade I & II 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. There are two known clades of Mpox now referred to as Clade I (former Congo Basin clade) and Clade II (former West African clade). Clade II consists of two subclades, IIa and IIb. Strains detected in the 2022 global outbreak belong to Clade IIb. Clade I leads to more severe illness and a higher rate of mortality. CDC reports show that some Clade I outbreaks have killed up to 10% of the people who get infected, while Clade II infection has less severe symptoms and more than 99.9% of people survive.

The RayBiotech Mpox Virus Clade I & II PCR Nucleic Acid Detection Kit is a ready to use PCR assay for the detection of both Clade I and Clade II Mpox virus DNA in a liquid sample. This kit uses two sets of specific primers and probes in different fluorescent channels to detect the C3L gene for Clade I and G2R gene for Clade II. Each set of primers and probes are designed to target the specific region in the genome where Clades I and II have multiple differing mutations so that the two strains can be differentiated. Primer and probe for internal control, RNase P are also integrated in the assay for quality validation.

### 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-Clades-MM | Buffer, dNTP's, enzyme, ROX reference dye | 1000 µL / tube | 1 tube |
| Primers and Probe Mix | PCR-MPXV-Clades-PP | MPXV G2R, C3L/RNase P Primer & Probe Mix | 500 µL / tube | 1 tube |
| Positive Control | PCR-MPXV-Clades-POS | Synthetic MPXV Clade I/II genomic DNA/RNase P Positive Control | 50 µL / tube | 1 tube |
| Negative Control | PCR-MPXV-Clades-NEG | Nuclease-free water | 500 µL / 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, pipettes, 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 methods, 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 may be 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 10 µL 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 |
|---|---|---|---|
| 2X PCR 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** | **20 µL** | **20 µL** | **20 µL** |

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 100 ng recommended) to appropriate wells and pipette up and down at least 5 times to mix.

3.3. Add 5 µL positive control template and 5 µL negative control template to appropriate wells.

3.4. Seal the plate or tubes tightly.

3.5. Centrifuge the plate or tubes for 30 seconds at a 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 “Clade II”, this channel will detect the G2R gene of Clade II MPXV. 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 “Clade I”, this channel will detect the C3L gene of Clade I MPXV. 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.

4.4. Save the file and run program. A sample image of PCR amplification is shown (Figure 1).

**Table 2: PCR Program**

| Step | | Temperature (°C) | Time | Temperature Ramp Rate | Number of Cycles |
|---|---|---|---|---|---|
| Stage 1 | Initial Denaturation | 95 | 5 min | 1.6°C/sec | 1 |
| Stage 2 | Denature | 95 | 5 s | 1.6°C/sec | 45 |
| | Anneal, extend, detect fluorescence | 61 | 20 s | 1.6°C/sec | |

5. **Result and Analysis**

The positive and negative control PCR reactions are considered valid if the 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 ≥ 40 or 2) the negative control has a Ct < 40. 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 Clade II MPXV signal on FAM channel and Clade I MPXV 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 |
|---|---|---|
| Clade II MPXV G2R | Ct < 40 | Ct ≥ 40 or no Amplification |
| Clade I MPXV C3L | Ct < 40 | Ct ≥ 40 or no Amplification |
| RNase P | Ct < 40 | Ct ≥ 40 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 | Clade I or II MPXV Ct | RNase P |
|---|---|---|
| + | < 40 | < 40 |
| - | No amplification, or Ct ≥ 40 | No amplification, or Ct ≥ 40 |

**Table 5. Interpretation of Sample Test**

| FAM (MPXV Clade II) | Cy5 (MPXV Clade I) | TAMRA (PCR validation) | PCR Result |
|---|---|---|---|
| - | + | + | Clade I + |
| - | + | - | Clade I + |
| + | - | + | Clade II + |
| + | - | - | Clade II + |
| - | - | + | 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.

*Note: The established Ct target cutoffs is listed here is based on a QuantStudio™ 5 Real-Time PCR machine (Thermo Fisher Scientific, Waltham, MA) and analyzed with Desing & Analysis Software 2.6.0. Each laboratory should establish their own cutoffs for the equipment and software in use.*

## PRODUCT PERFORMANCE INDEX

1. **Limit of Detection:** The LOD of the assay for MPXV Clade II is 5 copies/µL or 25 copies per reaction (5 µL of sample volume). The LOD of the assay for MPXV Clade I is 15 copies/µL or 75 copies per reaction (5 µL of sample volume).
2. **Repeatability:** Precision testing showed that the average coefficient of variation of the precision Ct values within this kit lot are ≤ 2.0%.

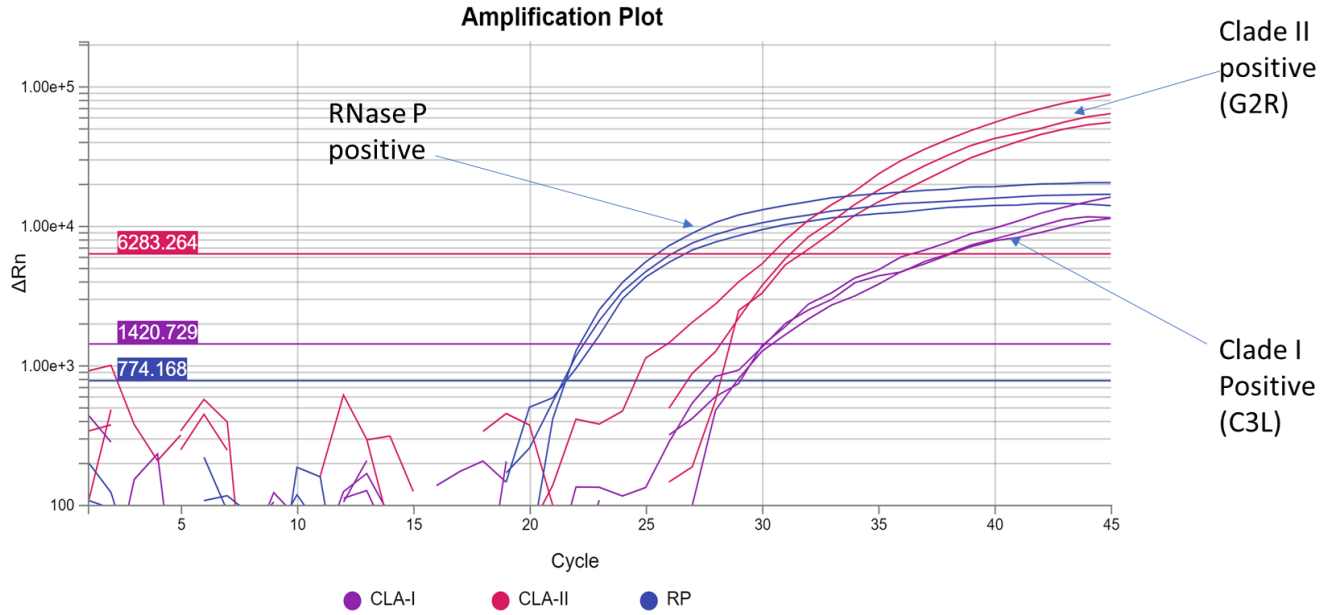


Figure 1. Positive control contains $10^2$ copies/µL of MPXV Clade II G2R Synthetic DNA (Red color, FAM channel), $10^2$ copies/µL of MPXV Clade I virus C3L Synthetic DNA (Purple color, Cy5 channel) and fixed amount of RNase P template (Blue color, TAMRA channel). 5 µL positive control were added to 15 µL of master mix and amplified in a QuantStudio™ 5 Real-Time PCR machine.