PCRassays.com

ASSAY NAME: POX1

Quantity: 100 x 20µL PCR reactions

3-Plex assay: Monkeypox and Orthopox viruses with human RPP30 DNA control

SKU: BUN-POX1-D-BR-100

(RUO). Research Use Only.
Not for use in Diagnostic Procedures.

INTRODUCTION

The MPX1 assay (for monkeypox virus) detects the gene for the "B21R Surface glycoprotein cadherin-like domain putative membrane-associated glycoprotein" (MPXVgp182, Protein ID: gb|URK20621.1). The MPX1 assay is specific for Monkeypox and will not detect most other orthopox viruses. The MPX1 assay has been experimentally verified (not shown) to NOT detect cowpox, horsepox, racoonpox, or ectromelia viruses (specificity experiments were performed on 500 BP synthetic constructs of the homologous B21R surface glycoprotein genes from each virus). Bioinformatics analysis of the homologous B21R surface glycoprotein of variola virus contains many mutations (i.e., 10 mutations in FP and RP regions) compared to monkeypox and thus variola is unlikely to be detected by the MPX1 assay.

The OPV1 assay (for orthopox viruses) detects the conserved protein coding family called "B4R Schlafen-like (Cop-B2R)" (MPXVgp165 Protein ID: gb|URK20603.1). The assay has been experimentally verified to detect synthetic constructs of: monkeypox, cowpox, horsepox, buffalopox, rabbitpox, ectromelia virus, akhmeta virus, and vaccinia virus. Experimental performance data shown below are for monkeypox only (please contact us if further information about OPV1 detection of other viruses is needed). The OPV1 assay was experimentally verified NOT to detect raccoonpox. Based upon a bioinformatic analysis of genome sequences of the variola virus (i.e., causative agent of smallpox), the OPV1 assay should NOT detect variola.

CONTENTS

The primers and probes in the POX1 assay are provided in Tube 1 as a 20X concentrated working solution that detects Monkeypox, Orthopox, and human RPP30 DNA, which serves as an extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Monkeypox	FAM	BHQ-1	1, 2
RPP30-DNA control	HEX	BHQ-1	3
Orthopox viruses	Cy5	BHQ-2	4, 5

The probes are designed as TaqMan[6] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

Assay contents:

Tube 1: Primer/Probe mix (20X) for Monkeypox virus, orthopox viruses, and human RPP30 DNA

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: Synthetic 500 bp DNA fragments of Monkeypox virus, orthopox virus, and hRPP30DNA.

Tube 3: InhibiTaq RT-qPCR enzyme Mastermix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.

ASSAY HANDLING

The POX1 bundle is shipped on ice, and should be stored at -20 °C. The tubes should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq qPCR enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water	7

Notes: To improve assay sensitivity, up to 9 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2. Molecular biology grade water (NOT included) should be used to prepare the PCR reactions.

A PCR protocol was used in-house for verification on a Bio-Rad CFX96™ Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 15 seconds*
4	Plate Read
5	Go to Step 2, repeat 44× more

For QuantStudio instruments, we recommend a Step 3 cycle time of 22 seconds at 55 °C.

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 38 cycles and final RFU >200 (BioRad) is considered "positive" or "+" in the Table below. Note: On QuantStudio 5, 6, 7, 12K instruments the final RFU cutoff is >200,000.

MPX1 FAM™	OPV1 Cy5™	RPP30 HEX™	Recommended Interpretation
–	–	–	The PCR reaction failed. Please repeat the experiment.
–	–	+	The sample doesn't contain monkeypox, cowpox, horsepox, buffalopox, rabbitpox, ectromelia virus, akhmeta virus, and vaccinia virus.
+	+	–	The sample contains monkeypox DNA. The sample may also contain cowpox, horsepox, buffalopox, rabbitpox, ectromelia virus, akhmeta virus, and vaccinia virus. The sample may not contain human DNA (hRPP30 gene Intron I).
+	+	+	The sample contains monkeypox DNA and human DNA (hRPP30 gene Intron I). The sample may also contain cowpox, horsepox, buffalopox, rabbitpox, ectromelia virus, akhmeta virus, and vaccinia virus.
–	+	–	The sample does NOT contain monkeypox DNA. The sample contains at least one of the following orthopox viruses: cowpox, horsepox, buffalopox, rabbitpox, ectromelia virus, akhmeta virus, and/or vaccinia virus. The sample does not contain human DNA (hRPP30 gene Intron I).
–	+	+	The sample does NOT contain monkeypox DNA. The sample contains human DNA (hRPP30 gene Intron I) and at least one of the following orthopox viruses: cowpox, horsepox, buffalopox, rabbitpox, ectromelia virus, akhmeta virus, and/or vaccinia virus.

VERIFICATION EXPERIMENTS

The POX1 assay verification was carried out as a 3-plex assay that simultaneously detects DNA from Monkeypox virus, orthopox viruses, and human RPP30 DNA, which serves as a positive control assay.

Experiments (**Figure 1**) were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the verification experiments contained 1×10^4 copies/reaction of synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes. The "monkeypos_construct1" is from the Monkeypox B21R Surface glycoprotein and should be detected only by MPX1 assay (FAM). The "orthopox_construct1" is from Monkeypox B4R Schlafen-like gene and only be detected by OPV1 assay (Cy5). Various experiments are also shown with added human RPP30 DNA gene, and human genomic DNA. Both MPX1 and OPV1 assays have also been experimentally verified to detect extracts of clinical monkeypox positive samples (data not shown).

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only target construct was added. The results show a limit of detection (LOD) <100 copies/reaction.

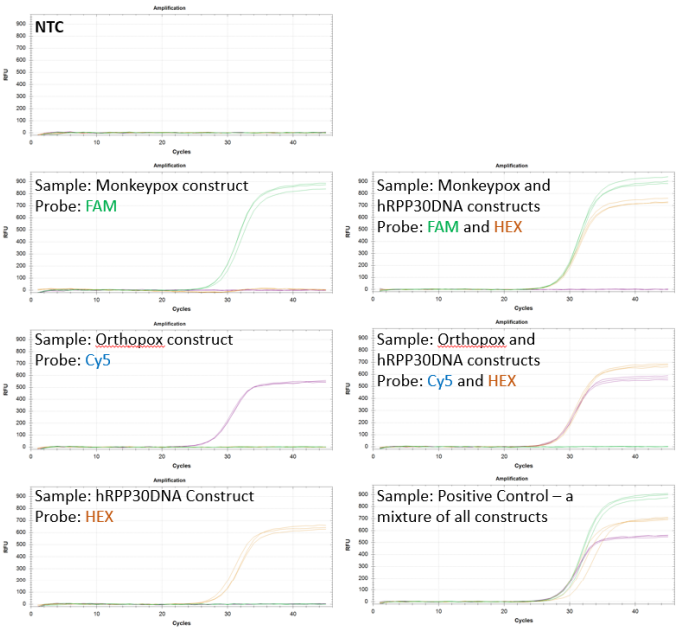

Figure 1: Verification experiments with targets (given in text boxes for each panel). The primers and probes of monkeypox orthopox, and RPP30DNA are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific.

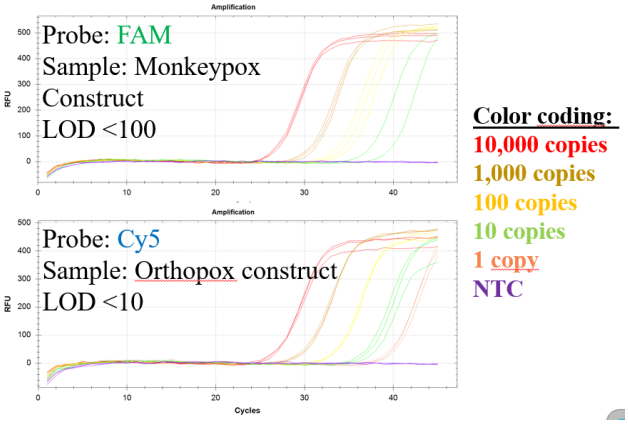

Figure 2: Serial dilution experiments show LOD <100 molecules for both targets.

Conclusion: The data in **Figure 1** indicate that the primers and probe of MPX1, OPV1, and hRPP30DNA are compatible with each other to detect monkeypox and other orthopox viruses in the matrix of human sample extract.

CONTACT US

For assistance, please contact DNA Software (the parent company of PCRassays.com) using the link: https://www.dnasoftware.com/contact/

Address: Michigan Life Science and Innovation Center,
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Phone: (734) 222-9080

NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corporation.

[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[3] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.

[4] Cy5™, a trademark of GE Healthcare.

[5] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[6] TaqMan™ is a trademark of Roche Diagnostics, Inc.