



Kit: OPV1-RPP30DNA

Quantity: 100 x 20µL PCR reactions

Orthopox virus with human RPP30 DNA control

Gene: B4R Schlafen-like protein

SKU: PNP-OPV1-D-100

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

INTRODUCTION

The assay OPV1-RPP30DNA is a quantitative polymerase chain reaction (qPCR) test intended for the detection of nucleic acid from several orthopox viruses and 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 has also been experimentally validated on extracts of clinical monkeypox positive samples (data not shown). 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. This kit is for research use only and should not be used for diagnostic procedures.

The performance of this kit was tested using a 500 BP synthetic DNA construct (monkeypox_construct1 DNA) from Twist Biosciences. The limit of detection (LOD) is below 10 copies/reaction.

CONTENTS

A mix of primers/probe targeting the orthopox virus is provided in a tube as a 20x concentrated working solution. The fluorophore of the probe is FAM™ (Carboxyfluorescein, a trademark of Life Technologies, Inc) and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.) A mix of primers/probe targeting human RPP30 DNA Intron I is also provided in the tube (20x concentrated) as a PCR positive control for human samples. The fluorophore of the probe is HEX™ (Hexachloro-fluorescein, a trademark of Life Technologies, Inc), and the quencher is BHQ-1™.

A mix of DNA constructs of monkeypox (OPV1_construct) and hRPP30_DNA is provided in a separate tube for a double positive control. The concentration of each DNA construct is 5,000 cps/µL.

KIT HANDLING AND CONTAMINATION

Kit contents:

- Tube 1: 20x Primers/Probe specific for orthopox and 20x Primers/Probe specific for human RPP30 gene Intron I.
- Tube 2: Double positive control (monkeypox (OPV1_construct) and hRPP30_DNA constructs at 5,000 cps/uL in 40 µL)

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

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq mastermix (2x)	10
OPV1-RPP30DNA mix (20x)	1
Sample	2
Water	7

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences.

A PCR protocol was used in-house for pre-validation 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 @ 60 °C for 30 seconds
4	Plate Read
5	Go to Step 2, repeat 44x more

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 is considered “positive” or “+” in the Table below.

OPV1 (FAM™)	RPP30 (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain orthopox DNA
+	–	The sample contains orthopox DNA. The sample may not contain human DNA (hRPP30 gene Intron I)

The kit OPV1-RPP30DNA is shipped at ambient temperature, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed.

+	+	The sample contains orthopox DNA and human DNA (hRPP30 gene Intron I)
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PRE-VALIDATION EXPERIMENTS

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the validation experiments include water NTC, a 10× diluted human genomic DNA, a water sample spiked with 10000 copies/uL of orthopox_construct1_DNA, and a 10× diluted human genomic DNA spiked with 10000 copies/uL of monkeypox_construct1_DNA. The results of these experiments are shown in **Figure 1** below:

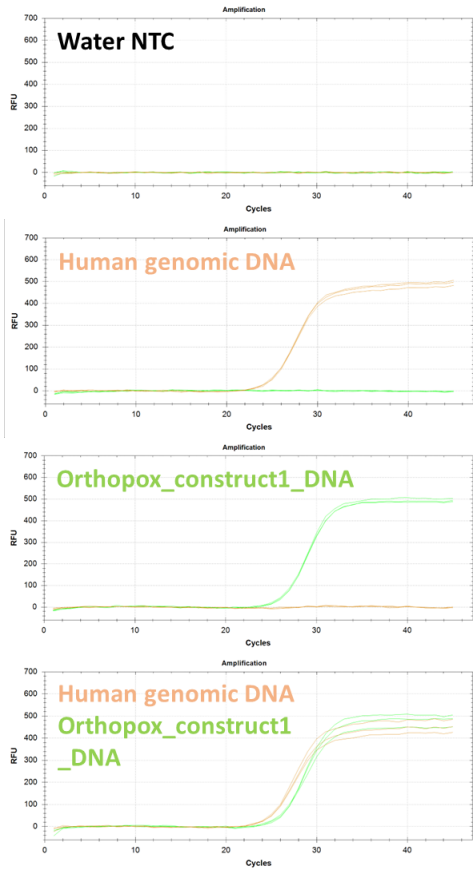
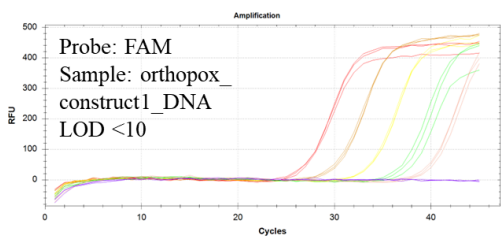


Figure 1: Validation experiments with targets (given in text boxes for each panel). Primers and probes for both OPV1 and RPP30DNA are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific. The **FAM** probe detects orthopox (orthopox_construct1_DNA). The **HEX** probe detects RPP30 gene Intron I (human genomic DNA).



Color coding:

10,000 copies
1,000 copies
100 copies
10 copies
1 copy
NTC

Figure 2: Serial dilution experiments show LOD <10 molecules for orthopox_construct1_DNA.

Conclusion: The data in **Figure 1** indicate that the OPV1 primers and probe are compatible with DNAS RPP30DNA positive control primers and probe in a 2-plexing application to detect orthopox in the matrix of human sample extract.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only the orthopox_construct1_DNA was added. The results show a limit of detection (LOD) <10 copies/reaction.

CONTACT US

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

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