

## Rabbitpox Virus Quantitative PCR (qPCR) Assay Detection Kit

### Catalog No. NR-35510

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### For research use only. Not for human use.

#### Contributor and Manufacturer:

BEI Resources

#### Product Description:

The Rabbitpox Virus Quantitative PCR Assay Detection Kit (NR-35510) is designed to detect and quantitate the presence of rabbitpox virus. The assay consists of the following components:

- 1) Probe designed with 6-carboxyfluorescein (6-FAM) at the 5' end and a non-fluorescent quenching dye at the 3' end (NR-26771)
- 2) Forward and reverse primers (NR-26769 and NR-26770, respectively)
- 3) Linearized plasmid-based standard containing rabbitpox virus sequences (NR-26776)

The plasmid-based standard, NR-26776, was designed by inverting every other 10 nucleotides within the target region, except for the primer and probe binding regions. This approach resulted in a positive control sequence with the same GC content and melting temperature as the original rabbitpox virus sequence, but one that facilitates discrimination of the presence of true rabbitpox material from false positives resulting from plasmid contamination.

The selected primers amplify a range of orthopoxviruses due to homology within the family, while the probe was designed to detect rabbitpox virus specifically. The assay has shown specificity for rabbitpox virus over vaccinia virus, monkeypox virus and cowpox virus. Preferential, but not specific, behavior was observed with swinepox virus, raccoonpox virus, tanapox virus, canarypox and pseudocowpox virus, all of which showed amplification only at or after 40 cycle thresholds.

Each kit contains enough probe, primer and plasmid-based standard for approximately 96 reactions using the assay protocol outlined in Appendix I.

#### Material Provided:

Each vial of primer contains 330  $\mu$ L in TE buffer (pH 8.0). Each vial of probe contains 200  $\mu$ L in TE buffer (pH 8.0). Each vial of plasmid-based standard contains  $4.27 \times 10^8$  copies per  $\mu$ L in 100  $\mu$ L TE buffer (pH 8.0). Lot-specific assay and component information is shown on the Certificate of Analysis.

#### Packaging/Storage:

Primers and probes were packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60 °C upon arrival. Freeze-

thaw cycles should be minimized. Probe samples should be kept in the dark at all times.

#### Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Rabbitpox Virus Quantitative PCR (qPCR) Assay Detection Kit, NR-35510."

#### Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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**APPENDIX I  
Quantitative PCR Assay for the Detection and Quantitation of Rabbitpox Virus**

**Recommended Reagents/Equipment**

Reagent/Equipment	Source	Catalog #
Rabbitpox Virus Quantitative PCR Probe	BEI Resources	NR-26771
Rabbitpox Virus Quantitative PCR Forward Primer	BEI Resources	NR-26769
Rabbitpox Virus Quantitative PCR Reverse Primer	BEI Resources	NR-26770
Plasmid Containing Rabbitpox Virus Sequences, Linearized	BEI Resources	NR-26776
SsoFast Probes Supermix 2X	BioRad	172-5230
Molecular Grade Water	ATCC®	60-2450
CFX 96-Well Plate Thermal Cycler	BioRad	184-5096

**Preparation of Plasmid-Based Standard Curve Samples**

Dilution Tube	Volume (µL)	Volume Molecular Grade Water (µL)	Concentration (Copies per 5 µL) <sup>1</sup>
Undiluted NR-26776	---	---	$2.14 \times 10^9$
1	5 of undiluted NR-26776	102	$1 \times 10^8$
2	5 of Tube 1	45	$1 \times 10^7$
3	5 of Tube 2	45	$1 \times 10^6$
4	5 of Tube 3	45	$1 \times 10^5$
5	5 of Tube 4	45	$1 \times 10^4$
6	5 of Tube 5	45	1000
7	5 of Tube 6	45	100
8	5 of Tube 7	45	10
9	5 of Tube 8	45	1

<sup>1</sup>See Certificate of Analysis, Table 4.

**Reaction Mix<sup>1</sup>**

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Grade H <sub>2</sub> O	N/A	6.5
SsoFast Probes Supermix	2X	10
Probe <sup>2,3</sup> - NR-26771	5 µM	0.5
Forward Primer <sup>2</sup> - NR-26769	10 µM	1.5
Reverse Primer <sup>2</sup> NR-26770	10 µM	1.5
Nucleic acid sample	N/A	5
		Total – 25 µL

<sup>1</sup>Reaction mix should be kept on bench-top cooler until ready for use.

<sup>2</sup>Primers and probe are supplied at working stock concentrations.

<sup>3</sup>6-carboxyfluorescein probe must be protected from light at all times.

**Cycling Protocol**

Cycle	# of Repeats	Step	Conditions
1	1	1	95.0°C for 2 minute
2	45	1	95.0°C for 15 seconds
		2	62.0°C for 45 seconds

**Instructions**

1. Prepare unknown nucleic acid samples.
2. This assay was developed using Bio-Rad reagents and detection system. Please refer to the CFX System Manual for information regarding plate and run setup.
3. When analyzing the data, especially the standard curve, it is important that the PCR efficiency fall between 80-120% and that the C<sub>T</sub> values are separated by approximately 3.3 cycles.