

## Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Assay Detection Kit

### Catalog No. NR-9351

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

#### Contributor and Manufacturer:

BEI Resources

#### Product Description:

The Monkeypox Virus Hemagglutinin (HA) Gene-Specific Quantitative PCR Assay Detection Kit (NR-9351) is designed to detect and quantitate the presence of monkeypox virus. The assay was developed using the hemagglutinin gene from monkeypox virus, Zaire 79 (NR-2324) and consists of the following components:

- 1) Probe designed with 6-carboxyfluorescein (6-FAM) at the 5' end and both the minor groove binder (MGB) and a non-fluorescent quenching dye at the 3' end (NR-9347)
- 2) Forward and reverse primers (NR-9348 and NR-9349, respectively)
- 3) Linearized plasmid-based standard containing an HA gene insert derived from monkeypox virus, Zaire 79 in a commercial vector (NR-4076)

#### Specificity for Monkeypox Virus over Non-Orthopoxviruses

This Monkeypox Virus HA Gene-Specific Quantitative PCR Assay has been shown to discriminate monkeypox viruses (Zaire 79 and WRAIR 7-61) from a variety of non-orthopoxviruses, non-viral samples and cell lines acquired from the ATCC® and BEI Resources collections. Quantitative PCR results using extracted nucleic acid were compared against cycle threshold ( $C_T$ ) values calculated from the serially-diluted plasmid-based standard, which routinely gave standard curves with correlation coefficient values of approximately 0.98.

#### Preferential Detection of Monkeypox Virus over other Orthopoxviruses

Although the HA genes of orthopoxviruses share over 95% homology, the Monkeypox Virus HA Gene-Specific Quantitative PCR Assay has been shown to preferentially detect monkeypox virus over other orthopoxviruses (vaccinia virus, cowpox virus, ectromelia virus, raccoonpoxvirus, rabbitpoxvirus) in the ATCC® and BEI Resources collections. When the copy number of orthopoxvirus DNAs are normalized using a Pan-Orthopox Virus Quantitative PCR Assay<sup>1</sup> (BEI Resources NR-9350) prior to quantitation using the Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Assay, monkeypox virus was detected between 35-fold and > 1000-fold more efficiently than all other orthopoxviruses tested.

Each kit contains enough probe, primer and plasmid-based standard for approximately 96 reactions using the assay protocol outlined in Appendix I. The primers, probe and plasmid-based standard are available individually by requesting the BEI Resources NR number.

#### Material Provided:

Each vial of primer and probe contains 90 to 100 µL in TE buffer (pH 7.0). Each vial of plasmid-based standard contains approximately 0.14 µg (lot 61374700 and lot 58073323) and 6.0 µg (lot 61823487) in TE buffer (pH 7.0). Lot-specific information for each assay detection kit component 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: Monkeypox Virus Hemagglutinin Gene-Specific Quantitative PCR Assay Detection Kit, NR-9351."

#### 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/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

#### Disclaimers:

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**References:**

1. Kulesh, D. A., et al. "Smallpox and Pan-Orthopox Virus Detection by Real-Time 3'-Minor Groove Binder TaqMan Assays on the Roche LightCycler and the Cepheid Smart Cycler Platforms." *J. Clin. Microbiol.* 42 (2004): 601-609. PubMed: 14766823.
2. Likos, A. M., et al. "A Tale of Two Clades: Monkeypox Viruses." *J. Gen. Virol.* 86 (2005): 2661–2672. PubMed: 16186219. GenBank: DQ011155.
3. Shchelkunov, S. N., E. V. Gavrilova, and I. V. Babkin. "Multiplex PCR Detection and Species Differentiation of Orthopoxviruses Pathogenic to Humans." *Mol. Cell. Probes* 19 (2005): 1-8. PubMed: 15652214.
4. Olson, V. A., et al. "Real-Time PCR System for Detection of Orthopoxviruses and Simultaneous Identification of Smallpox Virus." *J. Clin. Microbiol.* 42 (2004): 1940-1946. PubMed: 15131152.

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