Cowpox Virus Quantitative PCR (qPCR) Assay Detection Kit

Catalog No. NR-35518

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For research use only. Not for human use.

Contributor and Manufacturer:

BEI Resources

Product Description:

The Cowpox Virus Quantitative PCR Assay Detection Kit (NR-35518) is designed to detect and quantitate the presence of cowpox 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 (BHQ-1*plus*) at the 3' end (NR-26774)
2) Forward and reverse primers (NR-26772 and NR-26773, respectively)
3) Linearized plasmid-based standard containing cowpox virus B9R gene-specific sequences (NR-26775)

The plasmid-based standard, NR-26775, 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 cowpox virus B9R gene sequence, but one that facilitates discrimination of the presence of true cowpox material from false positives resulting from plasmid contamination.

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 200 µL in TE buffer (pH 8.0). Each vial of probe contains 200 µL in TE buffer (pH 8.0). Each vial of plasmid-based standard contains 2×10^{10} copies per vial in 100 µL 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 -60º 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: Cowpox Virus Quantitative PCR (qPCR) Assay Detection Kit, NR-35518."

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.

Disclaimers:

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

Recommended Reagents/Equipment

Reagent/Equipment	Source	Catalog #
Cowpox Virus Quantitative PCR Probe	BEI Resources	NR-26774
Cowpox Virus Quantitative PCR Forward Primer	BEI Resources	NR-26772
Cowpox Virus Quantitative PCR Reverse Primer	BEI Resources	NR-26773
Plasmid Containing Cowpox Virus B9R Gene Sequences, Linearized	BEI Resources	NR-26775
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)[1]
Undiluted NR-26775	---	---	1×10^9
1	5 of undiluted NR-26775	45	1×10^8
2	5 of Tube 1	45	1×10^7
3	5 of Tube 2	45	1×10^6
4	5 of Tube 3	45	1×10^5
5	5 of Tube 4	45	1×10^4
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

[1]See Certificate of Analysis, Table 4.

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Reaction Mix[1]

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Grade H_20	N/A	8.25
SsoFast Probes Supermix	2X	10
Probe[2,3] - NR-26774	5 µM	0.25
Forward Primer[2] - NR-26772	10 µM	0.75
Reverse Primer[2] NR-26773	10 µM	0.75
Nucleic acid sample	N/A	5
		Total – 25 µL

[1]Reaction mix should be kept on bench-top cooler until ready for use.
[2]Primers and probe are supplied at working stock concentrations.
[3]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	64.0°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_T values are separated by approximately 3.3 cycles.

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