Product Information Sheet for NR-9350

Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Assay Detection Kit

Catalog No. NR-9350

This reagent is the tangible property of the U.S. Government.

For research use only. Not for human use.

Contributor:

NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH

Product Description:

The Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Assay Detection Kit (NR-9350) is designed to detect and quantitate the presence of orthopox viruses. The assay was developed using a segment of the E9L gene from vaccinia virus, New York City Board of Health (NR-54) 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 (NRC-1325; available individually as NR-9344)
2) Forward and reverse primers (NRC-1326 and NRC-1327; available individually as NR-9345 and NR-9346, respectively)
3) Linearized plasmid-based standard containing a segment of the E9L gene derived from vaccinia virus in a commercial vector (NRC-1324; available individually as NR-9343)

The Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Assay (NR-9350) was adapted from the assay described by Kulesh et al., 2004.[1] The assay has been shown to detect all orthopoxviruses tested from the BEI Resources collection, including two strains of monkeypox virus (Zaire 79 and WRAIR 7-61), six strains of vaccinia virus [Modified Vaccinia Ankara, Lister (Elstree), IHD, Lederle-Chorioallantoic, New York City Board of Health and Western Reserve] and one strain of cowpox virus (Brighton Red). The assay did not detect a wide variety of non-orthopoxviruses, non-viral samples and cell lines in 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.

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 45 ng 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 ºC 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 the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Assay Detection Kit, NR-9350."

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, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government make any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Biodefense and Emerging Infections Research Resources Repository
800-359-7370
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
Fax: 703-365-2898
E-mail: contact@beiresources.org

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale. This material may be subject to third party patent rights.

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.

ATCC® is a trademark of the American Type Culture Collection.

Support Provided by NIAID

Biodefense and Emerging Infections Research Resources Repository
800-359-7370
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
Fax: 703-365-2898
E-mail: contact@beiresources.org

APPENDIX I
Quantitative PCR Assay for the Detection and Quantitation of Pan-Orthopox Virus

Recommended Reagents/Equipment

Reagent/Equipment	Source	Catalog #
Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Probe	BEI Resources	NRC-1325 (NR-9344)
Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Forward Primer	BEI Resources	NRC-1326 (NR-9345)
Pan-Orthopox Virus E9L Gene-Specific Quantitative PCR Reverse Primer	BEI Resources	NRC-1327 (NR-9346)
Plasmid Containing E9L Gene from Vaccinia Virus, New York City Board of Health, Linearized	BEI Resources	NRC-1324 (NR-9343)
iTaq DNA Polymerase Kit	Bio-Rad	170-8870
dNTP Mix	Bio-Rad	170-8874
TE, pH 7.0	Ambion®	AM9861
Molecular Grade Water	ATCC®	60-2450
0.2 mL 8-Tube strips Without Caps	BioRad	TBS-0201
Optical Flat 8-Cap Strips	BioRad	TCS-0803
iQ Real-Time PCR Plates	BioRad	223-9441
Microseal 'B' Adhesive Seals	BioRad	MSB-1001
iQ5 Multicolor Real-Time PCR Detection System	BioRad	170-9780

Preparation of Plasmid-Based Standard Curve Samples

Dilution Tube	Volume (µL)	Volume TE, pH 7.0 (µL)	Concentration (Molecules per 5 µL)[1]
Undiluted NR-9343	---	---	5×10^8
1	100 of undiluted NR-9343	400	1×10^8
2	50 of Tube 1	450	1×10^7
3	50 of Tube 2	450	1×10^6
4	50 of Tube 3	450	1×10^5
5	50 of Tube 4	450	1×10^4
6	50 of Tube 5	450	1000
7	50 of Tube 6	450	100
8	50 of Tube 7	450	10
9	50 of Tube 8	450	1

[1]See Certificate of Analysis, Table 4.

Biodefense and Emerging Infections Research Resources Repository
800-359-7370
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
Fax: 703-365-2898
E-mail: contact@beiresources.org

Reaction Mix[1]

Reagent	Stock Concentration	Volume per Reaction (μL)
Molecular Grade H_20	---	29.25
PCR Buffer	10X	5
$MgCl_2$	50 mM	6.5
dNTP Mix	10 mM each	1
Probe[2,3] - NRC-1325 (NR-9344)	5 μM	1
Forward Primer[2] - NRC-1326 (NR-9345)	10 μM	1
Reverse Primer[2] NRC-1327 (NR-9346)	10 μM	1
iTaq polymerase	5 units per μL	0.25
Nucleic acid sample	---	5
		Total – 50 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 1 minute
2	45	1	95.0 °C for 15 seconds
		2	63.0 °C for 15 seconds

Instructions

1. Prepare unknown nucleic acid samples. Samples used in development of this assay included DNA extracted from virus using the Qiagen QIAamp® Viral RNA Mini Kit following the manufacturer's instructions.
2. This assay was developed using Bio-Rad reagents and detection system. Please refer to the iQ5 Multicolor Real-Time PCR Detection 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 somewhere between 90-105% and that the C_T values are separated by approximately 3.3 cycles.

Biodefense and Emerging Infections Research Resources Repository
800-359-7370
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
Fax: 703-365-2898
E-mail: contact@beiresources.org