

Pan-*Rickettsia* Quantitative PCR (qPCR) Assay Detection Kit

Catalog No. NR-35520

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For research use only. Not for use in humans.

Contributor and Manufacturer:

BEI Resources

Product Description:

The Pan-*Rickettsia* Quantitative PCR Assay Detection Kit (NR-35520) is designed to detect and quantitate *Rickettsia* species. The assay consists of the following components:

Table 1: Pan-*Rickettsia* Quantitative PCR Assay Detection Kit Components

Catalog Number	Component	Description
NR-35354	Forward Primer	The forward primer pairs with NR-35355 to amplify a sequence of 135 nucleotides from the 16S ribosomal RNA gene of <i>Rickettsia</i> species.
NR-35355	Reverse Primer	The reverse primer pairs with NR-35354 to amplify a sequence of 135 nucleotides from the 16S ribosomal RNA gene of <i>Rickettsia</i> species.
NR-35356	Probe	The probe is designed with 6-carboxyfluorescein (6-FAM) at the 5' end and both a minor groove binding moiety (MGB) and a non-fluorescent quenching dye (NFQ) at the 3' end.
NR-35519	Plasmid Standard	The plasmid standard contains <i>Rickettsia prowazekii</i> ribosomal RNA (rRNA) gene sequences.

The plasmid-based standard, NR-35519, is linearized pIDTBlue containing a one kilobase synthetic DNA insert corresponding to the 16S rRNA gene of the Madrid E strain of *Rickettsia prowazekii*. A ten nucleotide sequence within the 135 base pair target amplicon is inverted to facilitate discrimination between the presence of authentic *Rickettsia* genetic material and false positives resulting from plasmid contamination. NR-35520 is expected to detect virtually all *Rickettsia* species owing to the high degree of conservation of the 16S rRNA gene within the genus, and of the primer and probe sequences in particular.

Each kit contains enough primer and probe for approximately 250 reactions using the assay protocol outlined in Appendix I. Lot-specific Certificates of Analysis for individual components are available upon request.

Material Provided:

NR-35520 consists of the four vials in Table 1. Each vial of plasmid-based standard contains 2×10^{10} molecules in 100 μ L nuclease-free water. Each vial of primer contains 200 μ L in

1 mM Tris, 0.01 mM EDTA, pH 8. Each vial of probe contains 200 μ L in 0.5 mM Tris, 0.005 mM EDTA, pH 8. For lot 63511773, each vial of primer contains 200 μ L in 0.2 mM Tris, 0.02 mM EDTA, pH 8 and each vial of probe contains 200 μ L in 0.5 mM Tris, 0.05 mM EDTA, pH 8.

Packaging/Storage:

Primers, probe, and standard were packaged aseptically in cryovials. The product is provided frozen on dry ice and should be stored at -20° C or colder 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: Pan-*Rickettsia* Quantitative PCR (qPCR) Assay Detection Kit, NR-35520."

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

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APPENDIX I: QUANTITATIVE PCR ASSAY FOR THE DETECTION & QUANTITATION OF RICKETTSIA SPECIES

Reagents Provided

Reagent/Equipment	Source	Catalog #
Pan- <i>Rickettsia</i> Quantitative PCR Probe	BEI Resources	NR-35356
Pan- <i>Rickettsia</i> Quantitative PCR Forward Primer	BEI Resources	NR-35354
Pan- <i>Rickettsia</i> Quantitative PCR Reverse Primer	BEI Resources	NR-35355
Plasmid Containing Pan- <i>Rickettsia</i> B9R Gene Sequences, Linearized	BEI Resources	NR-35519

Required Reagents/Equipment but not Provided

DNA Polymerase
Molecular Grade Water
96-Well Plate

Preparation of Plasmid-Based Standard Curve Samples

Dilution Tube	Volume (µL)	Volume Molecular Grade Water (µL)	Concentration (Copies per 5 µL) ¹
Undiluted NR-35519	N/A	N/A	1 × 10 ⁹
1	5 of undiluted NR-35519	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

Reaction Mix¹

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Grade H ₂ O	N/A	11.875
DNA Polymerase buffer	10X	2.5
DNA Polymerase	5 U per µL	0.125
dNTPs	10 mM	0.5
MgCl ₂	50 mM	3
Probe ^{2,3} NR-35356	5 µM	0.5
Forward Primer ² NR-35354	10 µM	0.75
Reverse Primer ² NR-35355	10 µM	0.75
Nucleic acid sample	N/A	5
		Total – 25 µL

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

²Primers and probe are supplied at working stock concentrations.

³6-carboxyfluorescein probe must be protected from light at all times.

Recommended Cycling Protocol¹

Cycle	# of Repeats	Step	Conditions
1	1	1	95.0°C for 3 minutes
2	40	1	95.0°C for 15 seconds
		2	60.8°C for 15 seconds

¹Refer to your specific machine for recommended cycling conditions.