

Certificate of Analysis for NR-35520

Pan-Rickettsia Quantitative PCR (qPCR) Assay Detection Kit

Catalog No. NR-35520

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

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

- 1) Forward and reverse primers (NR-35354 and NR-35355, respectively)
- 2) Probe 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-35356)
- 3) Plasmid-based standard containing *Rickettsia prowazekii* ribosomal RNA (rRNA) gene sequences (NR-35519)

Lot: 63511773 Manufacturing Date: 12JUN2014

TEST	SPECIFICATIONS	RESULTS
Quantitative PCR – Standard Curve (Figure 1) Correlation coefficient PCR efficiency Dilution separations (Ct values) Quantitative sensitivity	≥ 0.98 85 to 110% ~ 3.33 cycles Report results	0.99 86.9% ~ 3.68 cycles 100 molecules per reaction
Content (OD ₂₆₀) NR-35354, Lot 62694613 (forward primer) NR-35355, Lot 62694614 (reverse primer)	Report results Report results	0.45 0.43
Content (μg) NR-35354, Lot 62694613 (forward primer) NR-35355, Lot 62694614 (reverse primer)	Report results Report results	13.8 13.8
Content (pmol) NR-35354, Lot 62694613 (forward primer) NR-35355, Lot 62694614 (reverse primer) NR-35356, Lot 62694615 (probe)	Report results Report results Report results	2000 2000 1000
Concentration (μΜ) NR-35354, Lot 62694613 (forward primer) NR-35355, Lot 62694614 (reverse primer) NR-35356, Lot 62694615 (probe)	Report results Report results Report results	10 10 5
Plasmid-Based Standard (NR-35519, Lot 62694627) ¹ Agarose Gel Electrophoresis of Linearized Plasmid DNA ¹ Concentration of DNA Molecules Sequencing of 16S rRNA Gene (420 nucleotides)	Single band at ~ 4,100 bp Report results Report results	Single band at ~ 4,100 bp 2.0 × 10 ¹¹ molecules per mL (1.0 × 10 ⁹ molecules per 5 µL) Consistent with <i>Rickettsia</i> species

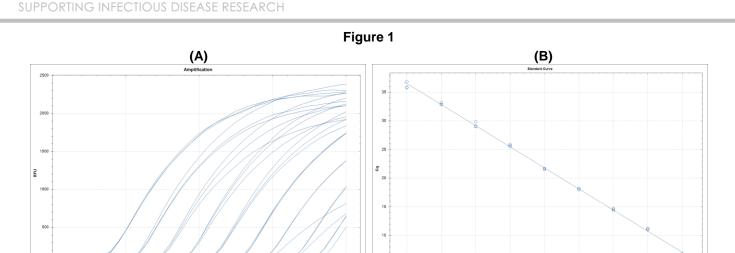
¹Plasmid DNA was extracted using a Plasmid Plus Maxi Kit (QIAGEN 12963). Purified plasmid DNA was linearized with *Eco*RI-HF (New England BioLabs, Inc. R3101).

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Representative quantitative PCR cycle graph (A) and associated standard curve (B). Data was obtained following the procedure in Appendix I of the Product Information Sheet for NR-35520 using the following reagents:

BEI Resources NR-35519, Lot 62694627 (Plasmid Containing 16S Ribosomal RNA Gene Sequences from *Rickettsia prowazekii*, Madrid E, Linearized)

BEI Resources NR-35520, Lot 62694613 (Pan-Rickettsia Quantitative PCR Forward Primer)

BEI Resources NR-35355, Lot 62694614 (Pan-Rickettsia Quantitative PCR Reverse Primer)

BEI Resources NR-35556, Lot 62694615 (Pan-Rickettsia Quantitative PCR Probe)

Date: 14 MAY 2015 Signature:

BEI Resources Authentication

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