Certificate of Analysis for NR-35520

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

Catalog No. NR-35520

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

Product Description:

NR-35220 is a Pan-*Rickettsia* Quantitative PCR (qPCR) Detection Assay to detect and quantitate *Rickettsia* species. The assay consists of the following 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 *Rickettsia* 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 *Rickettsia* 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 *Rickettsia prowazekii* ribosomal RNA (rRNA) gene sequences.

Lot: 70059015 Manufacturing Date: 24APR2023

TEST	SPECIFICATIONS	RESULTS
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System (Pre-vial; 9 replicates)	Report results	6.4×10^{12} genome copies/mL
Quantitative PCR – Standard Curve (Figure 1)		
Correlation coefficient	≥ 0.98	0.996
PCR efficiency	85 to 110%	88.8%
Dilution separations (C_t values)	~ 3.33 cycles	~ 3.62 cycles
Quantitative sensitivity	Report results	10 molecules/reaction
Concentration (µM)		
NR-35354 Lot 70059016 (forward primer)	Report results	10
NR-35355 Lot 70059017 (reverse primer)	Report results	10
NR-35356 Lot 70059018 (probe)	Report results	5
Plasmid-Based Standard (NR-35519 Lot 70059019)[1]		
Agarose Gel Electrophoresis of Linearized Plasmid DNA[1]	Single band at ~ 4,100 base pairs	Single band at ~ 4,100 base pairs
Concentration of DNA Molecules	Report results	2.0×10^{11} molecules/mL (1.0×10^{9} molecules/5 µL)

[1]Plasmid 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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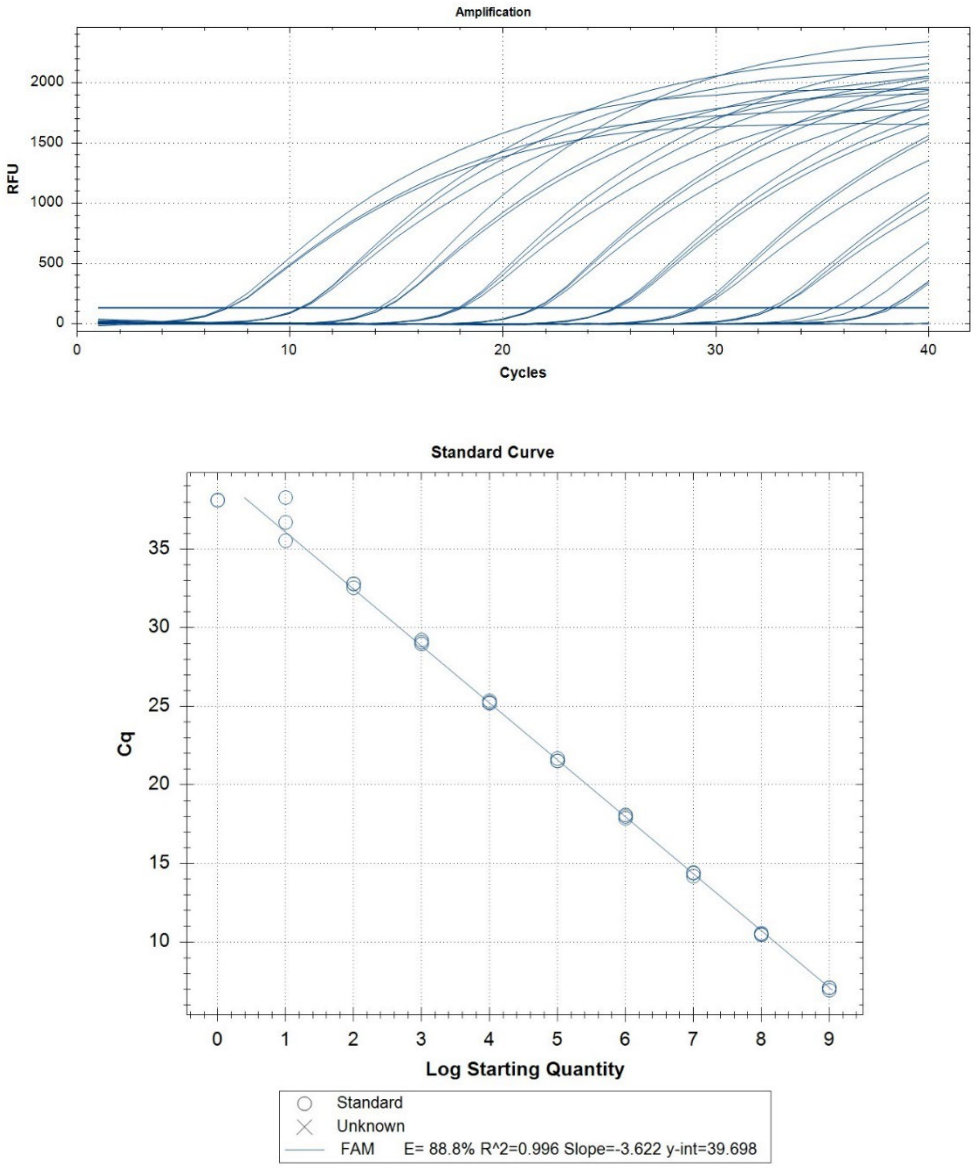
Figure 1: Quantitative PCR Cycle Graph (A) and Associated Standard Curve (B)



/Sonia Bjorum Brower/
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