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

## Catalog No. NR-35520

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

## For research use only. Not for human use.

### Contributor and Manufacturer:

BEI Resources

### 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)

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 *Ricekttsia 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:

Each vial of primer contains 200 µL in 0.2 mM Tris, 0.02 mM EDTA, pH 8.0. Each vial of probe contains 200 µL in 0.5 mM Tris, 0.05 mM EDTA, pH 8.0. Each vial of plasmid-based standard contains $2 \times 10^{10}$ molecules in 100 µL nuclease-free water. A representative assay is shown in Appendix II.

### Packaging/Storage:

Primers, probe, and standard were packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20º 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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.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 makes 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.

### 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.

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

Support Provided by NIAID

**BEI Resources**
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

# APPENDIX I
# Quantitative PCR Assay for the Detection and Quantitation of *Rickettsia* Species

## Recommended Reagents/Equipment

| Reagent/Equipment | Source | Catalog # |
|---|---|---|
| Pan-Rickettsia Quantitative PCR Probe | BEI Resources | NR-35356 |
| Pan-Rickettsia Quantitative PCR Forward Primer | BEI Resources | NR-35354 |
| Pan-Rickettsia Quantitative PCR Reverse Primer | BEI Resources | NR-35355 |
| Plasmid Containing Pan-Rickettsia B9R Gene Sequences, Linearized | BEI Resources | NR-35519 |
| iTaq™ DNA Polymerase | BioRad | 170-8870 |
| 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-35519 | --- | --- | $1 \times 10^9$ |
| 1 | 5 of undiluted NR-35519 | 45 | $1 \times 10^8$ |
| 2 | 5 of Tube 1 | 45 | $1 \times 10^7$ |
| 3 | 5 of Tube 2 | 45 | $1 \times 10^6$ |
| 4 | 5 of Tube 3 | 45 | $1 \times 10^5$ |
| 5 | 5 of Tube 4 | 45 | $1 \times 10^4$ |
| 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 Mix[1]

| Reagent | Stock Concentration | Volume per Reaction (µL) |
|---|---|---|
| Molecular Grade $H_20$ | N/A | 11.875 |
| iTaq™ buffer | 10X | 2.5 |
| iTaq™ DNA Polymerase | 5 U per µL | 0.125 |
| dNTPs | 10 mM | 0.5 |
| $MgCl_2$ | 50 mM | 3.0 |
| 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 |

[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.

**BEI Resources**
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

### Cycling Protocol

| 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 |

### Instructions

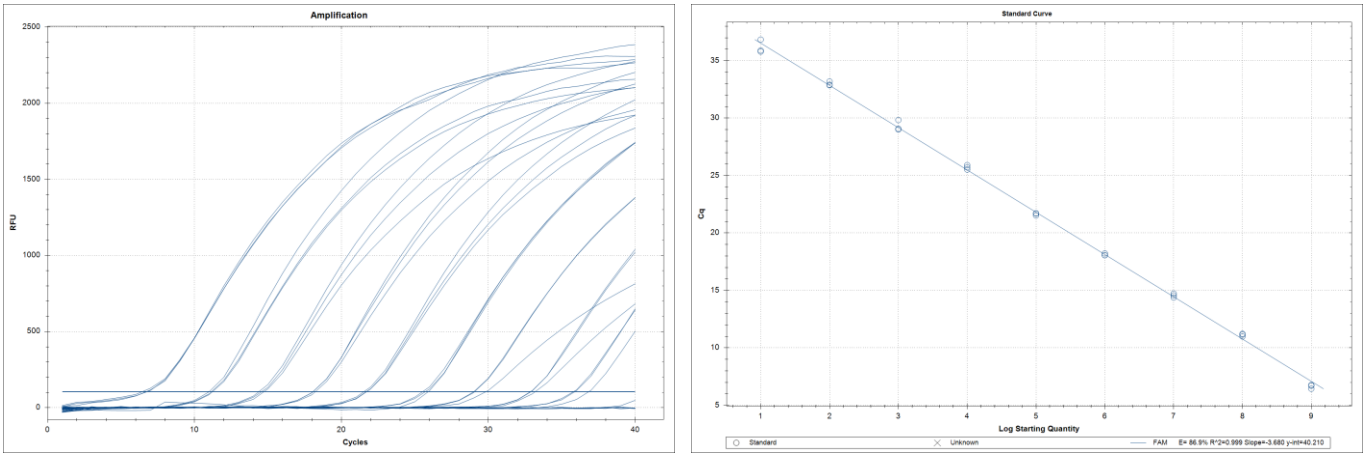
1. Prepare unknown nucleic acid samples.
2. This assay was developed using Bio-Rad reagents and detection system. Please refer to the CFX System Manual for information regarding plate and run setup.
3. When analyzing the data, especially the standard curve, it is important that the PCR efficiency fall between 80-120% and that the $C_T$ values are separated by approximately 3.3 cycles.

## APPENDIX II
## Representative Assay Results

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Quantitative PCR – Representative Standard Curve**[1] | | |
| Correlation coefficient | ≥ 0.98 | 0.99 |
| PCR efficiency | 85 to 110% | 86.9% |
| Dilution separations ($C_T$ values) | ~ 3.33 cycles | ~ 3.68 cycles |
| Quantitative sensitivity | Report results | 100 molecules per reaction |

[1]See Figure 1

### Figure 1




**(A)** **(B)**

**Representative quantitative PCR cycle graph (A) and associated standard curve (B) using NR-35520. The cycle threshold ($C_T$) was generated using regression analysis. Per-well baseline cycles have been determined automatically. The data analysis window is set at 95% of a cycle, centered at the end of the cycle.**

**BEI Resources**
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898