

**Dengue Virus Nucleic Acid Panel**

**Catalog No. NR-32847**

**Product Description:** The panel consists of nucleic acids extracted from dengue viruses, representing each of the four types. The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

**Lot: 70002377**

**NR-2771, Lot 64522915<sup>1</sup> (Dengue Virus Type 3 (DEN-3), Philippines/H87/1956; Manufactured 26SEP2016)**

TEST	SPECIFICATIONS	RESULTS
<b>Sequencing of Species-Specific Region (897 nucleotides)</b>	Consistent with DEN-3 Philippines/H87/1956	99% identity with DEN-3 Philippines/H87/1956 (GenBank: AB609590)
<b>Functional Activity by RT-PCR Amplification<sup>2</sup></b>	~ 1000 bp amplicon	~ 1000 bp amplicon (see Figure 1)
<b>Total RNA Content by RiboGreen<sup>®</sup> Measurement (Viral, Cellular, and Carrier)</b>	Report results	287 ng per 100 µL
<b>Viral RNA Content by Droplet Digital RT-PCR</b>	Report results	1.1 × 10 <sup>6</sup> copies per µL
<b>Virus Inactivation</b> 10% of total yield inoculated on Vero cells <sup>3</sup> and evaluated for immunofluorescence <sup>4,5</sup>	No viable virus detected	No viable virus detected

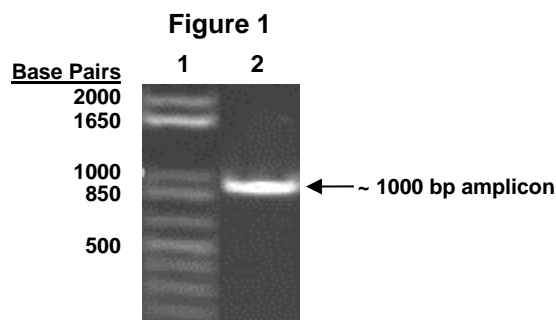
<sup>1</sup>Nucleic acid was extracted from a preparation of DEN-3, Philippines/H87/1956 (BEI Resources NR-80, Lot 64286309) using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

<sup>2</sup>Reverse transcription was performed using an iScript<sup>™</sup> cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-2771 in a 20 µL reaction; PCR was performed using iTaq<sup>™</sup> DNA Polymerase (Bio-Rad 170-8870) with 2 µL of cDNA in a 25 µL reaction.

<sup>3</sup>Vero cells: ATCC<sup>®</sup> CCL-81<sup>™</sup>

<sup>4</sup>Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)

<sup>5</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate dengue viruses as shown by the absence of cytopathic effect and viral antigen expression by indirect immunofluorescence after plating the entire extract on virus-susceptible cells.



NR-4287, Lot 63379480<sup>1</sup> (Dengue Virus Type 1 (DEN-1), Hawaii; Manufactured 20MAY2015)

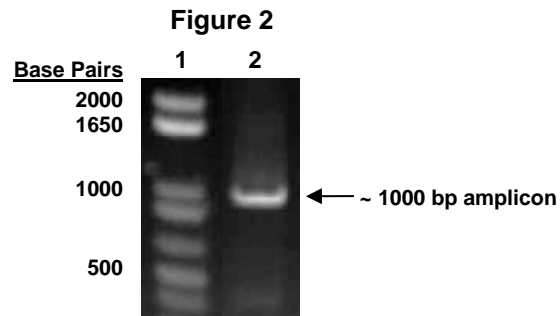
TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (918 nucleotides)	Consistent with DEN-1, Hawaii	100% identity with DEN-1, Hawaii (GenBank: EU848545)
Functional Activity by RT-PCR Amplification <sup>2</sup>	~ 1000 bp amplicon	~ 1000 bp amplicon (Figure 2)
Total RNA Content by RiboGreen <sup>®</sup> Measurement (Viral, Cellular, and Carrier)	Report results	531 ng per 100 µL
Virus Inactivation 10% of total yield inoculated on Vero cells <sup>3</sup> and evaluated for expression of viral antigen <sup>4</sup>	No viable virus detected	No viable virus detected

<sup>1</sup>Nucleic acid was extracted from a preparation of DEN-1, Hawaii (BEI Resources NR-82, Lot 58964764) using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

<sup>2</sup>Reverse transcription was performed using an iScript<sup>™</sup> cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-4287 in a 20 µL reaction; PCR was performed using iTaq<sup>™</sup> DNA Polymerase (Bio-Rad 170-8870) with 2 µL of cDNA in a 25 µL reaction.

<sup>3</sup>Vero cells: ATCC<sup>®</sup> CCL-81<sup>™</sup>.

<sup>4</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate 100% of dengue viruses, as shown by the absence of cytopathic effect (CPE) and expression of viral antigen after plating the entire extract on virus-susceptible cells



Lane 1: Invitrogen<sup>™</sup> TrackIt<sup>™</sup> 1 Kb Plus DNA Ladder  
 Lane 2: NR-4287

NR-4288, Lot 64152191<sup>1</sup> (Dengue Virus Type 2 (DEN-2), New Guinea C (NGC); Manufactured 29APR2016)

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (837 nucleotides)	Consistent with DEN-2, NGC	99% identity with DEN-2, NGC (GenBank: KM204118)
Functional Activity by RT-PCR Amplification <sup>2</sup>	~ 950 bp amplicon	~ 950 bp amplicon (Figure 3)
Total RNA Content by RiboGreen <sup>®</sup> Measurement (Viral, Cellular, and Carrier)	Report results	1.8 ng per 100 µL
Virus Inactivation 10% of total yield inoculated on LLC-MK2 <sup>3</sup> derivative cells and evaluated for immunofluorescence <sup>4,5</sup>	No viable virus detected	No viable virus detected

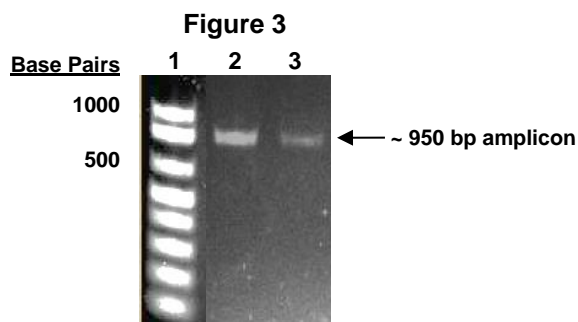
<sup>1</sup>Nucleic acid was extracted from a preparation of DEN-2, NGC (BEI Resources NR-84, Lot 62905413) using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

<sup>2</sup>Reverse transcription was performed using an iScript<sup>™</sup> cDNA Synthesis Kit (Bio-Rad 170-8890) with 10 µL of NR-4288 in a 20 µL reaction; PCR was performed using iTaq<sup>™</sup> DNA Polymerase (Bio-Rad 170-8875) with 2 µL of cDNA in a 25 µL reaction.

<sup>3</sup>LLC-MK2 derivative cells: ATCC<sup>®</sup> CCL-7.1<sup>™</sup>

<sup>4</sup>Using Anti-Dengue Virus Type II Antibody, clone 3H5-1 (Millipore MAB8702)

<sup>5</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate dengue viruses as shown by the absence of cytopathic effect and viral antigen expression by indirect immunofluorescence after plating the entire extract on virus-susceptible cells.



Lane 1: Invitrogen<sup>™</sup> TrackIt<sup>™</sup> 1 Kb Plus DNA Ladder  
 Lane 2: NR-4288, undiluted  
 Lane 3: NR-4288, 1:10 dilution

NR-4289, Lot 63719710<sup>1</sup> (Dengue Virus Type 4 (DEN-4), H241 (Tissue Culture Adapted); Manufactured 18JAN2016)

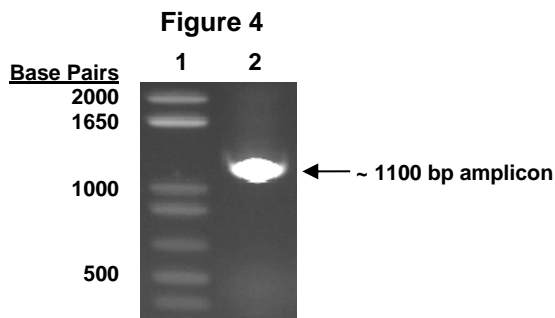
TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (1008 nucleotides)	Consistent with DEN-4, H241	99% identity with DEN-4, H241 (GenBank: AY947539)
Functional Activity by RT-PCR Amplification <sup>2</sup>	~ 1100 bp amplicon	~ 1100 bp amplicon (Figure 4)
Total RNA Content by RiboGreen <sup>®</sup> Measurement (Viral, Cellular, and Carrier)	Report results	123 ng per 100 µL
Virus Inactivation 10% of total yield inoculated on LLC-MK2 <sup>3</sup> derivative cells and evaluated for immunofluorescence <sup>4</sup>	No viable virus detected	No viable virus detected

<sup>1</sup>Nucleic acid was extracted from a preparation of DEN-4, H241 (BEI Resources NR-86, Lot 63069010) using a QIAamp<sup>®</sup> Viral RNA Mini Kit (Qiagen 52906).

<sup>2</sup>Amplified using iTaq<sup>™</sup> Universal SYBR<sup>®</sup> Green One-Step Kit (Bio-Rad 172-5151) with 5 µL of NR-4289 in a 40 µL reaction

<sup>3</sup>LLC-MK2 derivative cells: ATCC<sup>®</sup> CCL-7.1<sup>™</sup>

<sup>4</sup>Use of the QIAamp<sup>®</sup> Viral RNA Mini Kit has been demonstrated to consistently inactivate dengue viruses as shown by the absence of cytopathic effect and viral antigen expression by indirect immunofluorescence after plating the entire extract on virus-susceptible cells.



Lane 1: Invitrogen<sup>™</sup> TrackIt<sup>™</sup> 1 Kb Plus DNA Ladder  
Lane 2: NR-4289

Date: 14 FEB 2017

Signature: *Michael Q. Gynther*  
BEI Resources Authentication

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