Certificate of Analysis for NR-32847

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.

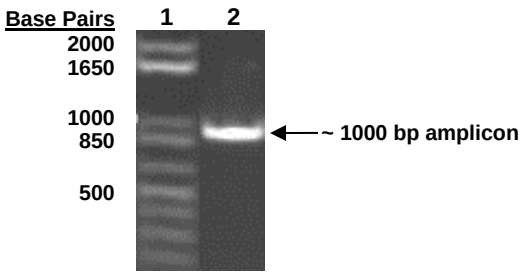
Lot: 70007480

NR-2771, Lot 64522915[1] (Dengue Virus Type 3 (DEN-3), Philippines/H87/1956; Manufactured 26SEP2016)

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

[1]Nucleic acid was extracted from a preparation of DEN-3, Philippines/H87/1956 (BEI Resources NR-80, Lot 64286309) using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).
[2]Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-2771 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 2 µL of cDNA in a 25 µL reaction.
[3]Vero cells: ATCC® CCL-81™
[4]Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)
[5]Use of the QIAamp® 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.

Figure 1



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-2771

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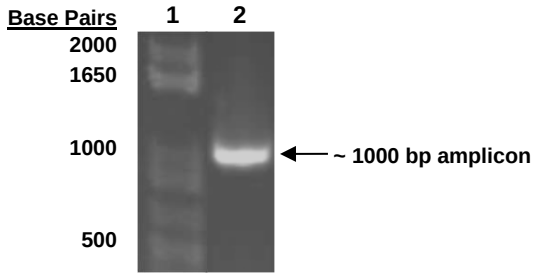
NR-4287, Lot 70002362[1] (Dengue Virus Type 1 (DEN-1), Hawaii; Manufactured 13FEB2017)

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (842 nucleotides)	Consistent with DEN-1, Hawaii Consistent with NR-82	100% identity with DEN-1, Hawaii (GenBank: EU848545) Consistent with NR-82
Functional Activity by RT-PCR Amplification[2]	~ 1000 bp amplicon	~ 1000 bp amplicon (Figure 2)
Total RNA Content by RiboGreen® Measurement (Viral, Cellular, and Carrier)	Report results	4.5 ng per 100 µL
Viral RNA Content by Droplet Digital RT-PCR	Report results	9.7×10^4 copies per µL
Virus Inactivation 10% of total yield inoculated on Vero cells[3] and evaluated for expression of viral antigen by indirect immunofluorescence[4,5]	No viable virus detected	No viable virus detected

[1]Nucleic acid was extracted from a preparation of DEN-1, Hawaii (BEI Resources NR-82, Lot 64218899) using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).
[2]Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-4287 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 2 µL of cDNA in a 25 µL reaction.
[3]Vero cells: ATCC® CCL-81™
[4]Using Anti-Dengue Virus Type I Antibody, clone 15F3-1 (Millipore MAB8701)
[5]Use of the QIAamp® 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.

Figure 2



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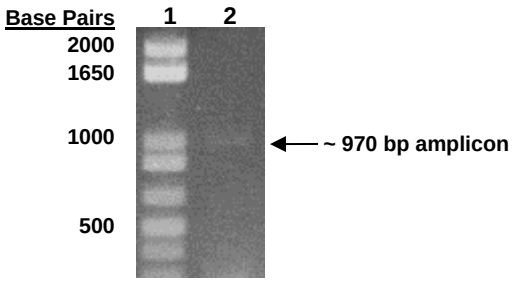
NR-4288, Lot 70004142[1] (Dengue Virus Type 2 (DEN-2), New Guinea C (NGC); Manufactured 06APR2017)

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (875 nucleotides)	Consistent with DEN-2, NGC Consistent with NR-84	99% identity with DEN-2, NGC (GenBank: KM204118) Consistent with NR-84
Functional Activity by RT-PCR Amplification[2]	~ 970 bp amplicon	~ 970 bp amplicon (Figure 3)
Total RNA Content by RiboGreen® Measurement (Viral, Cellular, and Carrier)	Report results	24.9 ng per 100 µL
Viral RNA Content by Droplet Digital RT-PCR	Report results	4.4×10^4 copies per µL
Virus Inactivation 10% of total yield inoculated on LLC-MK2[3] derivative cells and evaluated for expression of viral antigen by indirect immunofluorescence[4,5]	No viable virus detected	No viable virus detected

[1]Nucleic acid was extracted from a preparation of DEN-2, NGC (BEI Resources NR-84, Lot 64347312) using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).
[2]Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-4288 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 5 µL of cDNA in a 50 µL reaction.
[3]LLC-MK2 derivative cells: ATCC® CCL-7.1™
[4]Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)
[5]Use of the QIAamp® 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.

Figure 3



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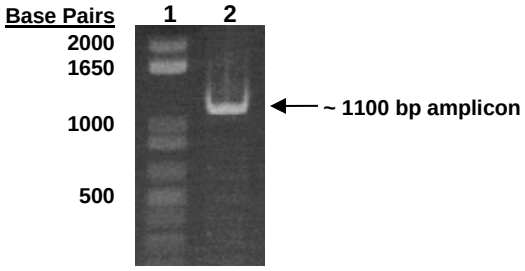
NR-4289, Lot 70004141[1] (Dengue Virus Type 4 (DEN-4), H241 (Tissue Culture Adapted); Manufactured 11APR2017)

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (987 nucleotides)	Consistent with DEN-4, H241	99% identity with DEN-4, H241 (GenBank: AY947539)
Functional Activity by RT-PCR Amplification[2]	~ 1100 bp amplicon	~ 1100 bp amplicon (Figure 4)
Total RNA Content by RiboGreen® Measurement (Viral, Cellular, and Carrier)	Report results	26.7 ng per 100 µL
Viral RNA Content by Droplet Digital RT-PCR	Report results	4.6×10^4 copies per µL
Virus Inactivation 10% of total yield inoculated on LLC-MK2[3] derivative cells and evaluated for expression of viral antigen by indirect immunofluorescence[4,5]	No viable virus detected	No viable virus detected

[1]Nucleic acid was extracted from a preparation of DEN-4, H241 (BEI Resources NR-86, Lot 64347313) using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).
[2]Reverse transcription was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad 170-8891) with 10 µL of NR-4289 in a 20 µL reaction; PCR was performed using iTaq™ DNA Polymerase (Bio-Rad 170-8870) with 5 µL of cDNA in a 50 µL reaction.
[3]LLC-MK2 derivative cells: ATCC® CCL-7.1™
[4]Using Anti-Dengue Virus Complex Antibody, clone D3-2H2-9-21 (Millipore MAB8705)
[5]Use of the QIAamp® 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.

Figure 4



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-4289

Date: 21 JUL 2017 **Signature:**

BEI Resources Authentication

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