Certificate of Analysis for NR-50548

Dengue Virus, Serotypes 1 to 4, Infected Cell Lysate, Gamma-Irradiated

Catalog No. NR-50548

Product Description: A crude preparation of C6/36 cells[1] infected with dengue viruses representing each of the four serotypes that was gamma-irradiated (5 x 10^6 RADs) on dry ice.

Lot[2,3]: 70003286 **Manufacturing Date: 09MAR2017**

TEST	SPECIFICATIONS	RESULTS
Pre-Inactivation Titer by $TCID_{50}$ Assay in C6/36[1] cells by IFA[4-6]	Report results	1.6 × 10^7 $TCID_{50}$ per mL
Viral Genome Copy Number by ddPCR[7]		
DEN-1, UIS 998	Report results	1.3 x 10^6 genome copies per µL
DEN-2, US/BID-V594/2006	Report results	6.8 x 10^2 genome copies per µL
DEN-3, US/BID-V1043/2006	Report results	8.1 x 10^4 genome copies per µL
DEN-4, UIS 497	Report results	1.6 x 10^4 genome copies per µL
Viral Genome Copy Number by qPCR		
DEN-1, UIS 998	Report results	9.2 x 10^5 genome copies per µL
DEN-2, US/BID-V594/2006	Report results	4.3 x 10^3 genome copies per µL
DEN-3, US/BID-V1043/2006	Report results	2.1 x 10^5 genome copies per µL
DEN-4, UIS 497	Report results	4.5 x 10^4 genome copies per µL
Amplification of DENV Sequence by RT-PCR		
DEN-1, UIS 998	Report results	~ 1010 base pair amplicon
DEN-2, US/BID-V594/2006	Report results	~ 1020 base pair amplicon
DEN-3, US/BID-V1043/2006	Report results	~ 1000 base pair amplicon
DEN-4, UIS 497	Report results	~ 1120 base pair amplicon
Virus Inactivation		
Cell culture safety test for residual virus[8]	No recovered virus	No recovered virus
NR-50548 was inoculated on C6/36 cells[1] and evaluated for cytopathic effect, viral antigen expression by indirect immunofluorescence assay, and presence of viral RNA by real-time RT-PCR after serial passage[9]	No viable virus detected	No viable virus detected

[1]C6/36 cells: *Aedes albopictus* mosquito larval epithelial cells (ATCC® CRL-1660™)
[2]Source of irradiated antigen: BEI Resources NR-49713 lot 70000743, NR-43280 lot 70000744, NR-43282 lot 70000746 and NR-49724 lot 70000745. Additional information about the individual components of NR-50548 can be found on the BEI website.
[3]All tests were completed post-inactivation unless otherwise specified
[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[5]14 days at 28°C and 5% CO_2
[6]Using Anti-Dengue Virus Complex Antibody (Millipore MAB8705).
[7]ddPCR data was obtained post-vial from 9 replicates for DEN-1, DEN-2, DEN-3 and 7 replicates for DEN-4 on the BioRad QX200 Droplet Digital PCR (ddPCR™) System
[8]Performed at University of Texas Medical Branch, Galveston, Texas, USA
[9]The inactivated virus preparation was plated on C6/36 cells and incubated for 14 days at 28°C and 5% CO_2; cell lysate and supernatant from these cultures was passaged to fresh monolayers of C6/36 cells and incubated for 14 days at 28°C and 5% CO_2.

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Certificate of Analysis for NR-50548

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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