b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-43280

Dengue Virus Type 2, DENV-2/US/BID-V594/2006

Catalog No. NR-43280

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

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval clone C6/36 cells[1] infected with dengue virus type 2, DENV-2/US/BID-V594/2006

Lot[2]: 62484838 **Manufacturing Date: 05MAY2014**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells[1]	Report results	Ballooning, rounding, and sloughing
Identification by Indirect Fluorescent Antibody (IFA) Assay[3]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (934 nucleotides)	Consistent with DENV-2/US/BID-V594/2006	99% identity with DENV-2/US/BID-V594/2006 (GenBank: EU482725)
Titer by $TCID_{50}$ Assay[4,5] in C6/36 Cells[1] with IFA Readout[3]	Report results	8.9×10^5 $TCID_{50}$ per mL
Functional Activity by RT-PCR Assay	~ 1 Kb amplicon	~ 1 Kb amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[6], 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)

[2]Grown in Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate (ATCC® 30-2002) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 6 days at 28°C with 5% CO_2.

[3]Using Anti-Dengue Virus Type II Antibody, clone 3H5-1 (Millipore MAB8702)

[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]8 days at 28°C and 5% CO_2

[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 29 SEP 2016 **Signature:** Michael R. Gyrla

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