b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52019

Recombinant Respiratory Syncytial Virus, A2 Expressing Red Fluorescent Protein (RFP) (rgRSV-BNI)

Catalog No. NR-52019

Product Description:

Recombinant respiratory syncytial virus, A2 expressing red fluorescent protein (RFP) (rgRSV-BNI) was developed using a historical strain of RSV, A2, originally isolated in the 1950s in the USA. NR-52019 lot 70059816 was produced by infecting *Homo sapiens* epithelial carcinoma cells (HEp-2; ATCC® CCL-23™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2003™) containing Earle's Balanced Salt Solution with 25 mM HEPES, supplemented with 10% fetal bovine serum, (ATCC® 30-2020™) for 3 days at 37°C with 5% CO_2.

Passage History:

Hep-2(6)/Hep-2(2) (The Ohio State University/BEI Resources)

Lot: 70059816 **Manufacturing Date: 30JUN2023**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells	Syncytia formation and cell disruption	Syncytia formation and cell disruption
Confirmation of RFP Expression	Fluorescence observed	Fluorescence observed
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® MiSeq™	≥ 98% identity with RSV, A2 (GenBank: KT992094.1)	99.99% identity with RSV, A2 (GenBank: KT992094.1)
Titer by $TCID_{50}$ Assay in HEp-2 Cells by Cytopathic Effect and Fluorescence (RFP)[1] (7 days at 37°C with 5% CO_2)	Report results	1.6×10^7 $TCID_{50}$/mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[2]	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, aerobic	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]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.
[2]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Technical Manager or designee, ATCC Federal Solutions

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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