b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49383

Influenza A Virus, A/WSN/33 (H1N1) PA-2A-NLuc (PASTN)

Catalog No. NR-49383

Product Description:

Influenza A virus, A/WSN/33 (H1N1) PA-2A-NLuc (PASTN) is a replication-competent influenza reporter virus that carries the luciferase variant NanoLuc® (NLuc) fused to the viral polymerase subunit A (PA). NR-49383 lot 70037384 was produced by infecting Madin-Darby Canine Kidney cells (MDCK; ATCC® CCL-34™) with influenza A virus, A/WSN/33 (H1N1) PA-2A-NLuc (PASTN) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 0.125% bovine serum albumin (Gibco 15260-037) and 1.0 µg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin for 6 days at 37°C and 5% CO_2.

Passage History:

C(3)/C(7) (Prior to deposit at BEI Resources/BEI Resources); C = MDCK cells

Lot: 70037384 **Manufacturing Date: 13AUG2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of PA-2A-NLuc Fusion Region (~ 760 nucleotides)	Identity confirmed	Identity confirmed[1]
Titer by $TCID_{50}$ Assay in MDCK Cells[2] (7 days at 37°C and 5% CO_2)	Report results	1.6×10^5 $TCID_{50}$ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[3]	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]Nucleotide sequencing confirmed the presence of the entire NanoLuc® ORF and the "self-cleaving" 2A peptide from porcine teschovirus fused in frame to the C-terminus of the modified PA gene, with the terminal 50 nucleotides of the native PA sequence repeated after the NLuc stop codon to restore native packaging sequences. See Tran, V., et al. "Highly Sensitive Real-Time *in vivo* Imaging of an Influenza Reporter Virus Reveals Dynamics of Replication and Spread." J. Virol. 87 (2001): 13321-13329 (PubMed: 24089552) for details.

[2]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 infectious titer (or infectivity) of a virus preparation.

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

/Heather Couch/
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Program 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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