

Certificate of Analysis for NR-43049

Influenza A Virus, A/swine/Manitoba/00446/2005 (H3N2)

Catalog No. NR-43049

Product Description: Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK) cells¹ infected with influenza A virus, A/swine/Manitoba/00446/2005 (H3N2)

Passage History: C2/C2 (Contributor/BEI Resources; C# = Number passages in MDCK cells)

Lot²: 63498604 Manufacturing Date: 30JUL2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK Cells ¹	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (670 nucleotides)	Consistent with A/swine/ Manitoba/00446/2005 (H3N2)	100% identity with A/swine/ Manitoba/00446/2005 (H3N2) (GenBank: CY159025)
Matrix (950 nucleotides)	Consistent with A/swine/ Manitoba/00446/2005 (H3N2)	99% identity with A/swine/ Manitoba/00446/2005 (H3N2) (GenBank: CY159026)
Titer by TCID ₅₀ Assay ^{3,4} in MDCK Cells ¹ With Direct Fluorescence Assay (DFA) Readout ⁵	Report results	$8.9 \times 10^5 \text{ TCID}_{50} \text{per mL}$
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic 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) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹MDCK; ATCC® CCL-34™

Date: 22 DEC 2015

Signature:

BEI Resources Authentication

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.

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²Grown in Eagle's Minimum Essential Medium (ATCC[®] 30-2003) supplemented with 0.3% bovine serum albumin (Invitrogen™ 15260-037) and 2.0 µg per mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin for 3 days at 37°C and 5% CO₂

³The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁴5 days at 37°C and 5% CO₂

⁵Using Light Diagnostics™ Influenza A Antibody FITC Reagent (Millipore 5017)

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