

Certificate of Analysis for NR-42939

Influenza A Virus, A/Georgia/M5081/2012 (H1N1)

Catalog No. NR-42939

Product Description: Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK) cells¹ infected with influenza A virus, A/Georgia/M5081/2012 (H1N1)

Passage History: H1/C6 (Contributor/BEI); H# = Number passages in human tracheobronchial epithelial cells; C# = Number passages in MDCK cells

Lot²: 62795203 Manufacturing Date: 13OCT2014

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK Cells ¹	Report results	Cell sloughing and rounding
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (388 nucleotides) Matrix (919 nucleotides)	Consistent with A/Georgia/ M5081/2012 (H1N1) Consistent with A/Georgia/ M5081/2012 (H1N1)	100% identity with A/Georgia/ M5081/2012 (H1N1) (GenBank: CY148267) 99% identity with A/Georgia/ M5081/2012 (H1N1) (GenBank: CY148172)
Titer by TCID ₅₀ Assay ^{3,4} in MDCK cells ¹	Report results	$1.6 \times 10^7 \text{ TCID}_{50} \text{ per mL}$
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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™

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²Grown in MDCK cells Eagle's Minimal Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg per mL sodium bicarbonate (ATCC[®] 30-2003) supplemented with 0.225% bovine serum albumin (InvitrogenTM 15260-037) and 2.0 μg per mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma-Aldrich[®] T1426) for 3 days at 33°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.

⁴7 days at 33°C and 5% CO₂

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Date: 03 MAR 2015

Title:

Technical Manager, BEI Authentication or designee

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