

Certificate of Analysis for NR-15268

Influenza A Virus, A/New York/18/2009 (H1N1)pdm09 (Tissue Culture Adapted)

Catalog No. NR-15268

Product Description: Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK) cells¹ infected with influenza A virus, A/New York/18/2009 (H1N1)pdm09

Passage History: E3/E2C4 (CDC/BEI); E# = Number passages in embryonated chicken eggs; C# = Number passages in MDCK cells

Lot²: 58690676 Manufacturing Date: 01AUG2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity using MDCK Cells ¹ Hemagglutination assay using cell lysate and supernatant from infected MDCK cells and 0.5% chicken red blood cells Cytopathic effect	Positive Report results	Positive Cell rounding and sloughing
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (444 nucleotides) Matrix (528 nucleotides)	Consistent with A/New York/18/2009 (H1N1) Consistent with A/New York/18/2009 (H1N1)	99% identity with A/New York/18/2009 (H1N1) (GenBank: GQ232064) 100% identity with A/New York/18/2009 (H1N1) (GenBank: GQ457504)
Titer by ID ₅₀ Assay ^{3,4,5} in MDCK cells	Report results	1.6 X 10 ⁶ TCID ₅₀ 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 Brucella 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

¹ATCC[®] CCL-34™

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²Grown in Eagle's Minimum Essential Medium (ATCC[®] 30-2003) supplemented with 10 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Affymetrix[®] USB[®] 22725), 10mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (Sigma-Aldrich[®] 83264) and 0.125% bovine serum albumin (Gibco[®] 15260-037) for 5 days at 35°C with 5% CO₂

³Determined by hemagglutination assay of culture supernatant using 0.5% chicken red blood cells

⁴The Infectious Dose 50% (CEID₅₀) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the inoculated host, 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 ID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁵6 days at 35°C with 5% CO₂

⁶Atlas, 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: 29 MAY 2012

Signature:

Title:

Technical Manager, BEI Authentication or designee

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