

Certificate of Analysis for NR-19845

Influenza A Virus, A/Netherlands/2290/2009 (H1N1)pdm09

Catalog No. NR-19845

Product Description: Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK)¹ infected with influenza A virus, A/Netherlands/2290/2009 (H1N1)pdm09

Passage History: tMk1C1/C2 (Depositor/BEI); tMK# = Number passages in tertiary monkey (*Macaca fascicularis*) kidney cells; C# = Number passages in MDCK cells

Lot²: 59525406 Manufacturing Date: 20APR2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK cells ¹	Report results	Cell rounding and sloughing
Identification by Hemagglutinin Gene Sequencing (430 nt)	Consistent with A/Netherlands/2290/2009 (H1N1)pdm09 (GenBank: CY065920)	Identical to A/Netherlands/2290/2009 (H1N1)pdm09 (GenBank: CY065920)
Identification by Matrix Gene Sequencing (891 nt)	Consistent with A/Netherlands/2290/2009 (H1N1)pdm09 (GenBank: CY065921)	Identical to A/Netherlands/2290/2009 (H1N1)pdm09 (GenBank: CY065921)
Titer by TCID ₅₀ Assay ^{3,4} in MDCK Cells ¹	Report results	2.8 × 10 ⁷ TCID ₅₀ 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 , aerobic	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: 09 FEB 2015 Signature: Michael Q. Gyptu

Title: Technical Manager, BEI Authentication or designee

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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BEI Resources www.beiresources.ora E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

²Grown in Dulbecco's Modified Eagle Medium (ATCC[®] 30-2002™) supplemented with 0.1% BSA (Invitrogen™ 15260-037) and 0.2 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma T1426-1G) for 2 days at 35°C and 5% CO₂

³The Tissue Culture Infectious Dose 50% (TCID₅₀) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the infected cells, 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.

⁴4 days at 35°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.