

Certificate of Analysis for NR-19805

Influenza A Virus, A/Wisconsin/629-D01313/2009 (H1N1)pdm09, Cell Isolate (Produced in Cells)

Catalog No. NR-19805

Product Description: Cell lysate and supernatant from primary Madin-Darby Canine Kidney (MDCK) cells¹ infected with influenza A virus, A/Wisconsin/629-D01313/2009 (H1N1)pdm09.

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

Lot²: 59525398 Manufacturing Date: 04APR2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK cells ¹	Report results	Refractile cell rounding and sloughing
Identification by Hemagglutinin Gene Sequencing (441 nt)	Consistent with A/Wisconsin/629-D01313/2009 (H1N1)pdm09 (GenBank: CY046539)	Identical to A/Wisconsin/629-D01313/2009 (H1N1)pdm09 (GenBank: CY046539)
Titer by TCID₅₀ Assay³,⁴ in MDCK Cells¹	Report results	2.8 X 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: 04 FEB 2015 Signature:

Title: Technical Manager, BEI Authentication or designee

Michael Q. Comple

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BEI Resources www.beiresources.org 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 3.8 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (USB 22725) for 5 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. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.