

Certificate of Analysis for NR-20344

Influenza A Virus, A/Brownsville/31H/2009 (H1N1)pdm09, Cell Isolate (Produced in Cells) Catalog No. NR-20344

Product Description: Cell lysate and supernatant from primary Rhesus monkey kidney cells (pRHMK)¹ infected with influenza A virus, A/Brownsville/31H/2009 (H1N1)pdm09.

Passage History: C1/C1 (Depositor/BEI); C# = Number passages in pRHMK cells

Lot²: 59548012 Manufacturing Date: 17FEB2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in pRHMK cells ¹	Report results	Refractile cell rounding and sloughing
Identification by Direct Fluorescent Antibody Assay ³	Report results	Fluorescence observed
Identification by Hemagglutinin Gene Sequencing (444 nt)	Consistent with A/Brownsville/31H/2009 (H1N1)pdm09 (GenBank: CY053214)	Identical to A/Brownsville/31H/2009 (H1N1)pdm09 (GenBank: CY053214)
Titer by TCID ₅₀ Assay ^{3,4,5} in pRHMK Cells ¹	Report results	1.6 X 10 ⁴ 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 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

¹pRHMK; Diagnostic Hybrids 49-T150A

Date: 10 FEB 2015 Signature: Millar Q. Com la

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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²Grown in Dulbecco's Modified Eagle Medium (ATCC[®] 30-2002™) supplemented with 0.1% BSA (Invitrogen™ 15260-037) and 0.01 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (USB 22725) for 10 days at 34°C and 5% CO₂

³Using Mouse Anti-Influenza A FITC-labeled Monoclonal Antibody (Millipore 5017)

⁴The 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.

⁵11 days at 34°C and 5% CO₂

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