SUPPORTING INFECTIOUS DISEASE RESEARCH

Influenza A Virus, A/Brownsville/39H/2009 (H1N1)pdm09, Cell Isolate (Produced in Cells)

Catalog No. NR-20346

Product Description: Cell lysate and supernatant from primary Rhesus monkey kidney cells (pRHMK)¹ infected with influenza A virus, A/Brownsville/39H/2009 (H1N1)pdm09.

Passage History: C1/C4 (Depositor/BEI); C# = Number passages in pRHMK cells

Lot²: 59548014

Manufacturing Date: 26JAN2011

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 (443 nt)	Consistent with A/Brownsville/39H/2009 (H1N1)pdm09 (GenBank: CY053277)	Identical to A/Brownsville/39H/2009 (H1N1)pdm09 (GenBank: CY053277)
Titer by TCID ₅₀ Assay ^{4,5} in pRHMK Cells ¹	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 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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

¹pRHMK; Diagnostic Hybrids 49-T150A

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

 5 6 days at 35°C and 5% CO₂

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

Date: 10 FEB 2015

Michael Q. Com he

Title:

Technical Manager, BEI Authentication or designee

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