

## **Certificate of Analysis for NR-20340**

## Influenza A Virus, A/Houston/3H/2009 (H1N1)pdm09, Cell Isolate (Produced in Cells)

Catalog No. NR-20340

**Product Description:** Cell lysate and supernatant from primary Rhesus monkey kidney cells (pRHMK)<sup>1</sup> infected with influenza A virus, A/Houston/3H/2009 (H1N1)pdm09.

Passage History: C1/C5 (Depositor/BEI); C# = Number passages in pRHMK cells

Lot<sup>2</sup>: 59548008 Manufacturing Date: 01FEB2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in pRHMK cells <sup>1</sup>	Report results	Refractile cell rounding and sloughing
Identification by Direct Fluorescent Antibody Assay <sup>3</sup>	Report results	Fluorescence observed
Identification by Hemagglutinin Gene Sequencing (444 nt)	Consistent with A/Houston/3H/2009 (H1N1)pdm09 (GenBank: CY052991)	99% identity with A/Houston/3H/2009 (H1N1)pdm09 (GenBank: CY052991)
Titer by TCID <sub>50</sub> Assay <sup>3,4,5</sup> in pRHMK Cells <sup>1</sup>	Report results	1.6 X 10 <sup>6</sup> TCID <sub>50</sub> per mL
Sterility (21-day incubation)  Harpo's HTYE broth <sup>6</sup> , 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 <sub>2</sub> , 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

<sup>&</sup>lt;sup>1</sup>pRHMK; Diagnostic Hybrids 49-T150A

**Date:** 10 FEB 2015 **Signature:** 

Title: Technical Manager, BEI Authentication or designee

Michael Q. Comple

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NR-20340\_59548008\_10FEB2015

<sup>&</sup>lt;sup>2</sup>Grown in Dulbecco's Modified Eagle Medium (ATCC<sup>®</sup> 30-2002<sup>™</sup>) supplemented with 0.1% BSA (Invitrogen<sup>™</sup> 15260-037) and 0.01 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (USB 22725) for 4 days at 34°C and 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>3</sup>Using Mouse Anti-Influenza A FITC-labeled Monoclonal Antibody (Millipore 5017)

<sup>&</sup>lt;sup>4</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the infectious titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>5</sup>10 days at 34°C and 5% CO<sub>2</sub>

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