

## **Certificate of Analysis for NR-13658**

## Influenza A Virus, A/California/04/2009 (H1N1)pdm09, Cell Isolate (Produced in Cells)

Catalog No. NR-13658

Product Description: Cell lysate and supernatant from Madin-Darby canine kidney cells (MDCK)<sup>1</sup>

infected with influenza A virus, A/California/04/2009 (H1N1)pdm09

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

Lot<sup>2</sup>: 58633728 Manufacturing Date: 21MAY2009

TEST	SPECIFICATIONS	RESULTS
Type/Subtype Identification and Purity by RT-PCR of RNA Influenza A virus (InfA) Influenza A virus, subtype H1 (human) Influenza A virus, subtype H3 (human) Influenza A virus, subtype H5 (human) Influenza A virus (swInfA) Influenza A virus (swH1) Influenza B virus	Detected None detected None detected None detected Detected Detected None detected	Detected None detected None detected None detected Detected Detected None detected
Identification by Hemagglutinin Gene Sequencing (1209 nt)	Homologous to A/California/04/2009 (H1N1)pdm09 (GenBank: GQ117044)	Homologous to A/California/04/2009 (H1N1)pdm09 (GenBank: GQ117044)
Hemagglutination Titer Using 0.5% Turkey Red Blood Cells	≥ 16	16
Titer by TCID <sub>50</sub> Assay <sup>3,4</sup> in MDCK Cells <sup>1</sup>	Report results	2.8 × 10 <sup>5</sup> TCID <sub>50</sub> per mL
Sterility (21-day incubation)  Harpo's HTYE broth <sup>5</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 Brucella agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	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>MDCK cells (ATCC<sup>®</sup> CCL-34™)

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<sup>&</sup>lt;sup>2</sup>Grown in Eagle's Minimum Essential Medium (ATCC<sup>®</sup> 30-2003) supplemented with 0.125% BSA (Invitrogen™ 15260-037), 10mM HEPES (Invitrogen™ 15630-080), and 2 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Signma-Aldrich<sup>®</sup> T1426) for 3 days at 33.5°C and 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>3</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>43</sup> days at 35°C and 5% CO2

<sup>&</sup>lt;sup>5</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



## **Certificate of Analysis for NR-13658**

Date: 14 JAN 2014 Signature: Doothy C. Young

Title: Technical Manager, BEI Authentication or designee

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