

## **Certificate of Analysis for NR-19810**

## Influenza A Virus, A/Wisconsin/629-D02452/2009 (H1N1)pdm09

Catalog No. NR-19810

**Product Description:** Cell lysate and supernatant from primary Madin-Darby Canine Kidney (MDCK) cells<sup>1</sup> infected with influenza A virus, A/Wisconsin/629-D02452/2009 (H1N1)pdm09.

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

Lot<sup>2</sup>: 59525403 Manufacturing Date: 20APR2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK cells <sup>1</sup>	Report results	Cell rounding and sloughing
Identification by Hemagglutinin Gene Sequencing (444 nt)	Consistent with A/Wisconsin/629-D02452/2009 (H1N1)pdm09 (GenBank: CY046587)	99% Identity to A/Wisconsin/629-D02452/2009 (H1N1)pdm09 (GenBank: CY046587)
Identification by Matrix Gene Sequencing (895 nt)	Consistent with A/Wisconsin/629-D02454/2009 (H1N1)pdm09 (GenBank: CY046588)	Consistent with A/Wisconsin/629-D02454/2009 (H1N1)pdm09 (GenBank: CY046588)
Titer by TCID <sub>50</sub> Assay <sup>3,4</sup> in MDCK Cells <sup>1</sup>	Report results	1.6 × 10 <sup>7</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 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>ATCC<sup>®</sup> CCL-34™

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<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 0.2 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma-Aldrich T1426-1G) for 2 days at 35°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>&</sup>lt;sup>4</sup>4 days at 35°C and 5% CO<sub>2</sub>

<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-19810**

Date: 04 FEB 2015 Signature:

Title: Technical Manager, BEI Authentication or designee

Michael Q. Comple

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