

## Certificate of Analysis for NR-15246

## Influenza A Virus, A/Tijuana/4/2009 (H1N1)pdm09, Cell Isolate (Produced in Cells)

Catalog No. NR-15246

**Product Description:** Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK) cells<sup>1</sup> infected with influenza A virus, A/Tijuana/4/2009 (H1N1)pdm09.

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

Lot<sup>2</sup>: 59462658 Manufacturing Date: 09APR2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK cells <sup>1</sup>	Report results	Refractile cell rounding and sloughing
Identification by Hemagglutinin Gene Sequencing (443 nt)	Consistent with Influenza A virus (H1N1)	Consistent with Influenza A virus (H1N1) <sup>3</sup>
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in MDCK Cells <sup>1</sup>	Report results	8.9 X 10 <sup>4</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>MDCK; ATCC<sup>®</sup> CCL-34

Date: 09 MAY 2013 Signature: Doothy C. Young

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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<sup>&</sup>lt;sup>2</sup>Grown in Dulbecco's Modified Eagle Medium (ATCC<sup>®</sup> 30-2002<sup>™</sup>) supplemented with 0.2% BSA (Invitrogen<sup>™</sup> 15260-037), 25 mM HEPES (Invitrogen<sup>™</sup> 15630-080) and 2.0 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (USB 22725) for 5 days at 35°C and 5% CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</sup>The nucleotide sequence obtained had 99% identity with numerous influenza A virus H1 HA sequences in the NCBI database; however, the HA gene sequence of A/Tijuana/4/2009 (H1N1)pdm09 has not been deposited at the time of document preparation.

<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>5 days at 35°C and 5% CO<sub>2</sub>

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