

## Certificate of Analysis for NR-28664

## Influenza A Virus, A/Wilson-Smith/1933-Mouse Adapted (H1N1) (clone)

Catalog No. NR-28664

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with influenza A virus, A/Wilson-Smith/1933–mouse adapted (H1N1)

Lot<sup>2</sup>: 62205669 Manufacturing Date: 19DEC2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs <sup>1</sup>		
Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Matrix Coding Regions		
Hemagglutinin (867 nucleotides)	Consistent with A/United Kingdom/1-MA/1933 (H1N1) <sup>3</sup>	99% identity with A/United Kingdom/1-MA/1933 (H1N1) (GenBank: CY090845) <sup>3</sup>
Matrix (943 nucleotides)	Consistent with A/United Kingdom/1-MA/1933 (H1N1) <sup>3</sup>	100% identity with A/United Kingdom/1-MA/1933 (H1N1) (GenBank: CY090846) <sup>3</sup>
Titer by CEID <sub>50</sub> Assay <sup>4,5</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	$2.8 \times 10^8$ CEID <sub>50</sub> per mL
Sterility (21-day incubation)		
Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

<sup>&</sup>lt;sup>1</sup>9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 08 MAY 2014 Signature:

**Title:** Technical Manager, BEI Authentication or designee

Michael Q. Com la

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 the allantoic cavity of embryonated chicken eggs<sup>1</sup> for 2 days at 35°C in a humidified chamber

<sup>&</sup>lt;sup>3</sup>NR-28664 was deposited to BEI Resources as A/Wilson-Smith/1933–Mouse Adapted (H1N1), but nucleotide sequence obtained from the same source material by the NIAID Influenza Genome Sequencing Consortium was deposited to NCBI as A/United Kingdom/1-MA/1933 (H1N1). This simply reflects a renaming of the original Wilson-Smith isolate using the modern standard nomenclature for influenza strains, specifying the geographical location of isolation and a sequential isolate number, with the addition of the MA notation to indicate adaptation to the murine host.

<sup>&</sup>lt;sup>4</sup>The Chicken Embryo Infectious Dose 50% (CEID₅₀) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the inoculated embryonated chicken eggs, 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 CEID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>5</sup>2 days at 35°C in a humidified chamber

<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.