

## **Certificate of Analysis for NR-3594**

Kilbourne F23: A/swine/Wisconsin/56/1976 (H1N1) Mutant, Low (L) Yield, Animal Isolate Catalog No. NR-3594

**Product Description:** Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with a low (L) yield mutant of influenza A virus, A/swine/Wisconsin/56/1976 (H1N1)

Lot<sup>2,3</sup>: 61982334 Manufacturing Date: 11SEP2013

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, Matrix, and Neuraminidase Coding Regions <sup>4</sup>		
Hemagglutinin (416 nucleotides)	Consistent with swine H1N1 influenza A virus	Consistent with swine H1N1 influenza A virus
Matrix (893 nucleotides)	Consistent with swine H1N1 influenza A virus	Consistent with swine H1N1 influenza A virus
Neuraminidase (396 nucleotides)	Consistent with swine H1N1 influenza A virus	Consistent with swine H1N1 influenza A virus
Titer by CEID <sub>50</sub> Assay <sup>5,6</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	2.8 × 10 <sup>6</sup> CEID <sub>50</sub> per mL
Sterility (21-day incubation)		
Harpo's HTYE broth <sup>7</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:** 04 DEC 2013

Signature: Mishael Q. Comba

Title: Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>Derived from NIAID Catalog No. V-331-0E5440

<sup>&</sup>lt;sup>3</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>4</sup>There is no sequence data for influenza A/swine/Wisconsin/56/1976 (H1N1) in the NCBI database; the sequences obtained for NR-3676 are consistent with those of H1N1 influenza viruses isolated from swine in North America in 1976 and 1977.

<sup>&</sup>lt;sup>5</sup>The Chicken Embryo Infectious Dose 50% (CEID<sub>50</sub>) 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the CEID<sub>50</sub> provides a measure of the infectious titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>6</sup>2 days at 35°C in a humidified chamber

<sup>&</sup>lt;sup>7</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.