

## **Certificate of Analysis for NR-3593**

Kilbourne F21: A/Wisconsin/3523/1988 (H1N1)

Catalog No. NR-3593

**Product Description:** Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with wild type influenza A virus, A/Wisconsin/3523/1988 (H1N1) (Kilbourne F21).

Lot<sup>2,3</sup>: 59628171 Manufacturing Date: 28JAN2011

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 Species- and Strain-Specific Regions		
Hemagglutinin (~ 448 nucleotides)	Consistent with influenza A virus (H1N1)	Consistent with influenza A virus (H1N1) <sup>4</sup>
Matrix gene (~ 893 nucleotides)	Consistent with A/Wisconsin/3523/1988 (GenBank: M63521)	99% identity with A/Wisconsin/3523/1988 (GenBank: M63521)
Titer by CEID <sub>50</sub> Assay <sup>5,6</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	8.9 X 10 <sup>6</sup> CEID <sub>50</sub> /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>10 to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

**Date:** 24 MAY 2011

Signature:

Title:

Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>Derived from NIAID Catalog No. V-331-0E5439

<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>The nucleotide sequence obtained had 96-99% identity with numerous influenza A virus H1 HA sequences in the NCBI database; however, the HA gene sequence of A/Wisconsin/3523/1988 has not been deposited.

<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. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.