

## **Certificate of Analysis for NR-28616**

## Influenza A Virus, A/duck/Wisconsin/480/1979 (H12N6) (uncloned)

Catalog No. NR-28616

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with influenza A virus, A/duck/Wisconsin/480/1979 (H12N6)

Lot<sup>2</sup>: 61660349 Manufacturing Date: 04APR2013

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 (375 nucleotides)  Matrix gene (953 nucleotides)	Consistent with A/duck/Wisconsin/480/1979 (H12N6)  Consistent with A/duck/Wisconsin/480/1979 (H12N6)	99% identity with A/duck/Wisconsin/480/1979 (H12N6) (GenBank: CY089645) 100% identity with A/duck/Wisconsin/480/1979 (H12N6) (GenBank: CY089646)
Titer by CEID <sub>50</sub> Assay <sup>3,4</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	$5.0 \times 10^7$ CEID <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>	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>9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania <sup>2</sup>Grown in the allantoic cavity of embryonated chicken eggs<sup>1</sup> for 3 days at 34°C in a humidified chamber

<sup>4</sup>3 days at 34°C in a humidified chamber

<sup>5</sup>Atlaś, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 08 AUG 2013 Signature:

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

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<sup>&</sup>lt;sup>3</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.