

## **Certificate of Analysis for NR-29029**

## Influenza A Virus, A/Puerto Rico/8-WG/1934 (H1N1)<sup>1</sup>

Catalog No. NR-29029

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>2</sup> infected with influenza A virus, A/Puerto Rico/8-WG/1934 (H1N1)

Lot<sup>3</sup>: 61502907 Manufacturing Date: 13FEB2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs <sup>2</sup> Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin Coding Region (1044 nucleotides)	Consistent with A/Puerto Rico/8-WG/1934 (H1N1)	100% identity with A/Puerto Rico/8-WG/1934 (H1N1) (GenBank: CY084006)
Titer by CEID <sub>50</sub> Assay <sup>4,5</sup> in Embryonated Chicken Eggs <sup>2</sup>	Report results	5.0 × 10 <sup>9</sup> CEID <sub>50</sub> /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>	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

Deposited and labeled as influenza A virus, A/Puerto Rico/8-34-WG/2010 (H1N1)

Signature: **Date:** 03 DEC 2013

> Title: Technical Manager, BEI Authentication or designee

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Fax: 703-365-2898

<sup>&</sup>lt;sup>2</sup>9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

<sup>&</sup>lt;sup>3</sup>Grown in the allantoic cavity of embryonated chicken eggs<sup>2</sup> for 2 days at 35°C in a humidified chamber

<sup>&</sup>lt;sup>4</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% (LD50) 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>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.