

## **Certificate of Analysis for NR-3666**

Kilbourne F150: A/USSR/90/1977 (HA, NA) x A/Puerto Rico/8/1934 (H1N1), Reassortant X-67

Catalog No. NR-3666

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with reassortant influenza A virus, A/USSR/90/1977 (HA, NA) x A/Puerto Rico/8/1934 (H1N1)

Lot<sup>2,3</sup>: 61190487 Manufacturing Date: 16AUG2012

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		
Hemagglutinin (446 nucleotides)	Consistent with A/USSR/90/1977 (H1N1)	100% identity with A/USSR/90/1977 (H1N1) (GenBank: HQ008265)
Matrix (924 nucleotides)	Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY105944)
Titer by CEID <sub>50</sub> Assay <sup>4,5</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	2.81 × 10 <sup>9</sup> 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 11-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 20 NOV 2012 Signature:

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

Milal Q. Comba

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<sup>&</sup>lt;sup>2</sup>Derived from NIAID Catalog No. V-331-0E5554

<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 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>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.