

**Kilbourne F-180: A/Shanghai/11/1987 (HA, NA) x A/Puerto Rico/8/1934 (H3N2),  
Reassortant-Mutant X-99 I-**

**Catalog No. NR-3487**

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with reassortant-mutant influenza A virus, A/Shanghai/11/1987 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)

**Lot<sup>2,3</sup>: 60341171**

**Manufacturing Date: 17NOV2011**

| TEST   | SPECIFICATIONS   | RESULTS  |
|--|--|--|
| <b>Identification by Infectivity Using Embryonated Chicken Eggs<sup>1</sup></b><br>Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells   | Positive   | Positive   |
| <b>Sequencing of Hemagglutinin and Matrix Coding Regions</b><br>Hemagglutinin (617 nucleotides)<br><br>Matrix (882 nucleotides)  | Consistent with A/Shanghai/11/1987 (H3N2)<br><br>Consistent with A/Puerto Rico/8/1934 (H1N1) | 99% identity with A/Shanghai/11/1987 (H3N2) (GenBank: CY121309) <sup>4</sup><br>100% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY033578) |
| <b>Titer by CEID<sub>50</sub> Assay<sup>5,6</sup> in Embryonated Chicken Eggs<sup>1</sup></b>  | Report results   | 8.9 × 10 <sup>8</sup> CEID <sub>50</sub> per mL  |
| <b>Sterility (21-day incubation)</b><br>Harpo's HTYE broth <sup>7</sup> , 37°C and 26°C, aerobic<br>Trypticase soy broth, 37°C and 26°C, aerobic<br>Sabouraud broth, 37°C and 26°C, aerobic<br>Blood agar, 37°C, aerobic<br>Blood agar, 37°C, anaerobic<br>Thioglycollate broth, 37°C, anaerobic<br>DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub> | No growth<br>No growth<br>No growth<br>No growth<br>No growth<br>No growth<br>No growth      | No growth<br>No growth<br>No growth<br>No growth<br>No growth<br>No growth<br>No growth  |
| <b>Mycoplasma Contamination</b><br>Agar and broth culture (14-day incubation at 37°C)<br>DNA detection by PCR of extracted Test Article nucleic acid   | None detected<br>None detected   | None detected<br>None detected   |

<sup>1</sup>10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

<sup>2</sup>Derived from NIAID Catalog No. V-331-0E5003

<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>4</sup>The hemagglutinin gene sequence obtained for NR-3487 contains a six nucleotide gap relative to the sequence of influenza A/Shanghai/11/1987 (H3N2) found in the NCBI database (CY121309). This gap corresponds to the deletion of amino acids S157 and E158 reported to be present in X-99 I- (Kilbourne, E. D., et al. "Influenza A Virus Haemagglutinin Polymorphism: Pleiotropic Antigenic Variants of A/Shanghai/11/87 (H3N2) Virus Selected as High Yield Reassortants." *J. Gen. Virol.* 74 (1993): 1311-1316. PubMed: 8336120).

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

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

## Certificate of Analysis for NR-3487

**Date:** 16 AUG 2012

**Signature:** 

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

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