

**Kilbourne F168: A/Mississippi/1/85 (HA, NA) x A/Puerto Rico/8/34 (H3N2), Reassortant X-87**

**Catalog No. NR-3502**

**Product Description:** Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with reassortant influenza A virus, A/Mississippi/1/85 (HA, NA) x A/Puerto Rico/8/34 (H3N2) (Kilbourne F168; X-87).

**Lot<sup>2,3</sup>: 58253967**

**Manufacturing Date: 18JUL2008**

| 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 Species-Specific Region (~ 880 nucleotides)</b>   | Influenza A virus   | Influenza A virus   |
| <b>Titer by CEID<sub>50</sub> Assay<sup>4,5</sup> in Embryonated Chicken Eggs<sup>1</sup></b>  | Report results  | 2.81 X 10 <sup>9</sup> CEID <sub>50</sub> /mL   |
| <b>RT-PCR Assay of Extracted RNA<sup>6</sup></b>   | ~ 1030 bp amplicon  | ~ 1030 bp amplicon  |
| <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>Sheep blood agar, 37°C, aerobic<br>Sheep 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 to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

<sup>2</sup>Derived from NIAID Catalog No. V-331-0E4844

<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 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>5</sup>2 days at 35°C in a humidified chamber

<sup>6</sup>The primers are described in Hoffmann, E., et al. "Universal Primer Set for the Full-Length Amplification of All Influenza A Viruses." *Arch. Virol.* 146 (2001): 2275-2289. PubMed: 11811679.

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

**Date:** 29 OCT 2008

**Signature:** Signature on File

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

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