

**Kilbourne F49: A/equine/Milan/2/63 (HA) x A/Texas/13/68 (NA) (H3N2)**

**Catalog No. NR-3606**

**Product Description:** Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with reassortant influenza A virus, A/equine/Milan/2/63 (HA) x A/Texas/13/68 (NA) (H3N2) (Kilbourne F49).

**Lot<sup>2,3</sup>: 58312178**

**Manufacturing Date: 22AUG2008**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity Using Embryonated Chicken Eggs<sup>1</sup></b> Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
<b>Sequencing of Species-Specific Region (420 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	8.89 X 10 <sup>7</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> Harpo's HTYE broth <sup>7</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> 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>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-331-0E5484A

<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: 07 NOV 2008**

**Signature: Signature on File**

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

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