

**Kilbourne F48: A/equine/Milan/2/1963 (HA) x A/Puerto Rico/8/1934 (NA) (H3N1)**

**Catalog No. NR-3603**

**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/2/1963 (HA) x A/Puerto Rico/8/1934 (NA) (H3N1)

**Lot<sup>2,3</sup>: 61487280**

**Manufacturing Date: 10JAN2013**

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 Hemagglutinin, Matrix, and Neuraminidase Coding Regions</b> Hemagglutinin (671 nucleotides)  Matrix (963 nucleotides)  Neuraminidase (530 nucleotides)	Consistent with A/equine-2 (H3N8) influenza virus <sup>4</sup>  Consistent with A/Puerto Rico/8/1934 (H1N1)  Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/equine/Miami/1/1963 (H3N8) (GenBank: CY028836) <sup>4</sup>  99% identity with A/Puerto Rico/8/1934 (H1N1) (Gen Bank: CY105897)  99% identity with A/Puerto Rico/8/1934 (H1N1) (Gen Bank: CY105898)
<b>Titer by CEID<sub>50</sub> Assay<sup>5,6</sup> in Embryonated Chicken Eggs<sup>1</sup></b>	Report results	1.1 × 10 <sup>8</sup> CEID <sub>50</sub> per mL
<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 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 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-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

<sup>2</sup>Derived from NIAID Catalog No. V-331-0E5481

<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>There is no sequence information for influenza A virus, A/equine//Milan/2/1963 available in the NCBI database or any of the various influenza databases. The hemagglutinin gene sequence obtained for NR-3603 is consistent with the published sequences of several influenza A (H3N8) strains isolated during the 1963 equine epizootic. It is most similar to influenza A virus, A/equine//Miami/1/1963 (GenBank: CY028836).

<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-3603

**Date:** 10 MAY 2013

**Signature:** 

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

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