

## **Certificate of Analysis for NR-3619**

Kilbourne F104: A/Rockefeller Institute/5/1957 (HA) x A/NWS/1934 (NA) (H2N1), Reassortant X-27

Catalog No. NR-3619

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with reassortant influenza A virus, A/Rockefeller Institute/5/1957 (HA) x A/NWS/1934 (NA) (H2N1)

Lot<sup>2,3</sup>: 58253971 Manufacturing Date: 25JUL2008

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 Species-Specific Region (880 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID <sub>50</sub> Assay <sup>4,5</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	8.89 × 10 <sup>6</sup> CEID <sub>50</sub> /mL
RT-PCR Assay of Extracted RNA <sup>6</sup>	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (21-day incubation)  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
Mycoplasma Contamination  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>&</sup>lt;sup>1</sup>10- to 11-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

**Date:** 24 OCT 2013

Signature:

Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>Derived from NIAID Catalog No. V-331-0E5501

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

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