

Certificate of Analysis for NR-3618

Kilbourne F103: A/Rockefeller Institute/5/57 (HA, NA) x A/equine/Prague/1/56 (H2N2) x A/NWS/34, Reassortant X-26

Catalog No. NR-3618

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A Virus, A/Rockefeller Institute/5/57 (HA, NA) x A/equine/Prague/1/56 (H2N2) x A/NWS/34 (Kilbourne F103; X-26).

Lot^{2,3}: 58479739 Manufacturing Date: 24JUL2009

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination (HA) assay using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species-Specific Region (~ 920 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	8.9 X 10 ⁸ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA ⁶	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 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 Brucella agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	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

¹9 to 11 day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania.

Date: 08 DEC 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5500.

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber in ambient atmosphere.

⁴The Chicken Embryo Infectious Dose 50% (CEID₅₀) 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the CEID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁵48 hours at 35°C in a humidified chamber

⁶The 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.

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