

Certificate of Analysis for NR-3611

Kilbourne F44: A/Port Chalmers/1/73 (HA) x A/equine/Prague/1/56 (NA) (H3N7), Reassortant Catalog No. NR-3611

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/Port Chalmers/1/73 (HA) x A/equine/Prague/1/56 (NA) (H3N7) (Kilbourne F44).

Lot^{2,3}: 59089233 Manufacturing Date: 19MAR2010

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species-Specific Region Matrix gene (~ 888 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID₅₀ Assay⁴₅⁵ in Embryonated Chicken Eggs¹	Report results	1.6 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 Sheep blood 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

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

Date: 21 JUN 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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NR-3611 59089233 21JUN2010

²Derived from NIAID Catalog No. V-331-0E5490

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 48 hours at 35°C in a humidified chamber

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.

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