

Certificate of Analysis for NR-3628

Kilbourne F126: A/England/864/1975 (HA, NA) x A/Puerto Rico/8/1934 (H3N2), Reassortant X-49

Catalog No. NR-3628

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/England/864/1975 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)

Lot^{2,3}: 60500175 Manufacturing Date: 04NOV2011

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 Hemagglutinin and Matrix Coding Regions Hemagglutinin (589 nucleotides) Matrix (814 nucleotides)	Consistent with A/England/864/1975 (H3N2) Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/England/864/1975 (H3N2) (GenBank: EF626614) 100% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY105944)
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	2.8 × 10 ⁹ CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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 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 Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 16 JUL 2012

Signature:

Title:

Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5510.

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

 $^{^4}$ The 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

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.