

Certificate of Analysis for NR-3672

Kilbourne F89: A/turkey/Massachusetts/3740/1975 (HA) x A/Nanchang/933/1995 (NA) x A/Puerto Rico/8/1934 (H6N2), Reassortant X-126

Catalog No. NR-3672

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/turkey/Massachusetts/3740/1975 (HA) x A/Nanchang/933/1995 (NA) x A/Puerto Rico/8/1934 (H6N2)

Lot^{2,3}: 61271606 Manufacturing Date: 11OCT2012

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, Matrix and Neuraminidase Coding Regions		
Hemagglutinin (303 nucleotides)	Consistent with A/turkey/Massachusetts/3740/ 1965 (H6N2) ⁴	99% identity with A/turkey/Massachusetts/3740/ 1965 (H6N2) (GenBank: CY087752) ⁴
Matrix (951 nucleotides)	Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/Puerto Rico/8/1934 (H1N1) (Gen Bank: CY105897)
Neuraminidase (470 nucleotides)	Consistent with A/Nanchang/933/1995 (H3N2)	99% identity with A/Nanchang/933/1995 (H3N2) (GenBank: CY112791)
Titer by CEID ₅₀ Assay ^{5,6} in Embryonated Chicken Eggs ¹	Report results	8.9×10^7 CEID ₅₀ per mL
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 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

¹⁰⁻day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

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²Derived from NIAID Catalog No. V-331-0E5560

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

⁴There is no sequence information for influenza A virus, A/turkey/Massachusetts/3740/1975 (H6N2) in the NCBI database or any of the various influenza databases. The hemagglutinin gene sequence obtained for NR-3672 is 99% identical to the published sequence of influenza A virus, A/turkey/Massachusetts/3740/1965 (H6N2). A review of the literature suggests that these two strain designations likely represent the same virus isolate, and that the progenitor of reassortant X-126 was originally isolated in 1965.

⁵The 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.

⁶2 days at 35°C in a humidified chamber

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



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Date: 14 FEB 2013 Signature: Millard Q. Cympla

Title: Technical Manager, BEI Authentication or designee

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