

Certificate of Analysis for NR-3644

Kilbourne F179: A/Shanghai/11/1987 (HA) x A/Leningrad/360/1986 (NA) x A/Puerto Rico/8/1934 (H3N2), Reassortant X-99b

Catalog No. NR-3644

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/Shanghai/11/1987 (HA) x A/Leningrad/360/1986 (NA) x A/Puerto Rico/8/1934 (H3N2)

Lot^{2,3}: 60341194 Manufacturing Date: 21OCT2011

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 (684 nucleotides)	Consistent with A/Shanghai/11/1987 (H3N2)	99% identity with A/Shanghai/11/1987 (H3N2) (GenBank: L19415)
Matrix (863 nucleotides)	Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: EF467824)
Neuraminidase (455 nucleotides)	Consistent with A/Leningrad/360/1986 (H3N2)	99% identity with A/Leningrad/360/1986 (H3N2) (GenBank: DQ508851)
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	5.0 × 10 ⁸ CEID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth No growth	No growth No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO ₂	No growth	No growth
Mycoplasma Contamination	None detected	None detected
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

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²Derived from NIAID Catalog No. V-331-0E5530

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days 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

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



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Date: 30 APR 2012

Signature: Derothy C. Young

Title:

Technical Manager, BEI Authentication or designee

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