

Certificate of Analysis for NR-3588

Kilbourne F100: A/Ulan Ude/01/2000 (HA, NA) x A/Puerto Rico/8/1934 (H3N2), Reassortant X-143

Catalog No. NR-3588

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/Ulan Ude/01/2000 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)

Lot^{2,3}: 60113487 Manufacturing Date: 22JUL2011

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 (687 nucleotides) Matrix (887 nucleotides)	Consistent with H3N2 influenza A virus ⁴ Consistent with A/Puerto Rico/8/1934 (H1N1)	Consistent with H3N2 influenza A virus ⁴ 100% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY033578)
Titer by CEID ₅₀ Assay ^{5,6} in Embryonated Chicken Eggs ¹	Report results	8.9×10^8 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

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

Date: 29 MAY 2012

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Title: Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5433

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

⁴The H3 HA sequence of influenza A/Ulan Ude/01/2000 is not in the NCBI database; the HA sequence obtained for NR-3588 is consistent with those of H3N2 influenza viruses isolated in 1999 and 2000.

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.