

Certificate of Analysis for NR-3520

Kilbourne F24: A/turkey/Kansas/4880/1980 (H) (H1N1), Mutant, High Yield, Avian Isolate

Catalog No. NR-3520

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with (Kilbourne F24) influenza A virus, A/turkey/Kansas/4880/1980 (H) (H1N1).

Lot^{2,3}: 59735812 Manufacturing Date: 04FEB2011

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- and Strain-Specific Regions Hemagglutinin gene (~ 425 nucleotides) Matrix gene (~ 887 nucleotides)	Consistent with A/turkey/Kansas/4880/1980 (GenBank: EU742636) Consistent with A/turkey/Kansas/4880/1980 (GenBank: EU742637)	99% identical to A/turkey/Kansas/4880/1980 (GenBank: EU742636) Identical to A/turkey/Kansas/4880/1980 (GenBank: EU742637)
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	8.9 X 10 ⁹ CEID ₅₀ /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 Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 24 MAY 2011

Signature: Dorothy C. Young

Title:

Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5028

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.