

Certificate of Analysis for NR-3470

Kilbourne F27:

A/turkey/Kansas/4880/1980 (HA, NA) x A/Puerto Rico/8/1934 (H1N1), H negative

Catalog No. NR-3470

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with H negative reassortant influenza A virus, A/turkey/Kansas/4880/1980 (HA, NA) x A/Puerto Rico/8/1934 (H1N1)

Lot^{2,3}: 61982336 Manufacturing Date: 19SEP2013

| 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 (444 nucleotides) Matrix (893 nucleotides) | Consistent with A/turkey/Kansas/4880/1980 (H1N1) Consistent with A/Puerto Rico/8/1934 (H1N1) | 99% identity with A/turkey/Kansas/4880/1980 (H1N1) (GenBank: EU742636) 100% identity with A/Puerto Rico/8/1934 (H1N1) (GenBank: CY033578) |
| Neuraminidase (446 nucleotides) | Consistent with A/turkey/Kansas/4880/1980 (H1N1) | 99% identity with A/turkey/Kansas/4880/1980 (H1N1) (GenBank: EU742638) |
| 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 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-331-0E4151

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



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Date: 05 DEC 2013 Signature: Mishael Q. Gyy hu

Title: Technical Manager, BEI Authentication or designee

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