

Certificate of Analysis for NR-3558

Kilbourne F181: A/duck/Singapore/645/1997 (H5N3), Wild Type

Catalog No. NR-3558

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A Virus, A/duck/Singapore/645/1997 (H5N3)

Lot^{2,3}: 58077631 Manufacturing Date: 15FEB2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination (HA) assay using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species-Specific Region (~ 910 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	1.6 × 10 ⁸ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA ⁶	~ 1030 bp amplicon	~ 1030 bp amplicon
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 Sheep blood agar, 37°C, aerobic Sheep 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

Date: 21 OCT 2013

Signature:

Title:

Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5271

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

⁶BM-M1 and BM-M-1027R primers; Obenauer, J. C., et al. "Large-Scale Sequence Analysis of Avian Influenza Isolates." <u>Science</u> 311 (2006): 1576-1580. PubMed: 16439620.

⁷Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.