

Certificate of Analysis for NR-3552

Kilbourne F79: A/turkey/Massachusetts/3740/75 (HA) x A/Beijing/32/92 (NA) x A/Puerto Rico/8/34 (H6N2), Reassortant X-118a

Catalog No. NR-3552

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/turkey/Massachusetts/3740/75 (HA) x A/Beijing/32/92 (NA) x A/Puerto Rico/8/34 (H6N2). (Kilbourne F79; X-118a).

Lot^{2,3}: 58555146 Manufacturing Date: 13MAR2009

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-Specific Region Matrix gene (~ 450 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	8.9 X 10 ⁸ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA ⁶	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (BacT/ALERT® 3D Microbial Detection System) 14-day incubation of NR-3552: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	No growth No growth	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: 20 MAY 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

800-359-7370

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²Derived from NIAID Catalog No. V-331-0E5240

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

⁶The primers are described in Hoffmann, E., et al. "Universal Primer Set for the Full-Length Amplification of All Influenza A Viruses." <u>Arch. Virol.</u> 146 (2001): 2275-2289. PubMed: 11811679.