

Certificate of Analysis for NR-9694

Influenza A Virus, A/Wisconsin/67/05 (HA, NA) x A/Puerto Rico/8/34 (H3N2), Reassortant X-161B

Catalog No. NR-9694

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza, A/Wisconsin/67/05 (HA, NA) x A/Puerto Rico/8/34 (H3N2), Reassortant X-161B.

Lot^{2,3}: 58155273 Manufacturing Date: 25APR2008

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 (623 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	8.89 X 10 ⁶ 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 to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

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²Source virus for this lot was prepared in embryonated hen eggs and provided by the Centers for Disease Control and Prevention, Atlanta, Georgia ³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber without CO₂

⁴The Chicken Embryo Infectious Dosé 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.

⁵48 hours at 35°C in a humidified chamber without CO₂

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.

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



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Date: 15 DEC 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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