

Certificate of Analysis for NR-9692

Influenza A Virus, A/New Caledonia/20/99 (HA, NA) x A/Puerto Rico/8/34 (H1N1), Reassortant X-139 (Kilbourne F98)

Catalog No. NR-9692

This reagent is the tangible property of the U.S. Government.

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A virus, A/New Caledonia/20/99 (HA, NA) x A/Puerto Rico/8/34 (H1N1), Reassortant X-139 (Kilbourne F98).

Lot^{2,3}: 58155270 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	Hemagglutination activity	Hemagglutination activity
Infected embryonated chicken eggs	Report results	No death of embryo
Sequencing of Species-Specific Region	Consistent with influenza A virus	Consistent with influenza A virus
Titer by CEID₅₀ Assay⁴₅⁵ in Embryonated Chicken Eggs¹	Report results	1.58 X 10 ⁷ CEID ₅₀ /mL
Functional Activity by RT-PCR Assay ⁶	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

¹⁰⁻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.

³Growth 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.

⁵48 hours 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.

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



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Date: 12 JAN 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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