Certificate of Analysis for NR-3567

Kilbourne F96: A/Japan/305/57 (HA, NA) x A/Puerto Rico/8/34 (H2N2), Reassortant X-135

Catalog No. NR-3567

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs[1] infected with influenza A Virus, A/Japan/305/57 (HA, NA) x A/Puerto Rico/8/34 (H2N2), (Kilbourne F96; X-135).

Lot[2,3]: 58079927 **Manufacturing Date: 22FEB2008**

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 (~ 800 nucleotides)	Influenza A virus	Influenza A virus
Titer by $CEID_{50}$ Assay[4,5] in Embryonated Chicken Eggs[1]	Report results	1.6 X 10^9 $CEID_{50}$/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	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

[1]9 to 11 day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania.
[2]Derived from NIAID Catalog No. V-331-0E5315.
[3]Grown in the allantoic cavity of embryonated chicken eggs[1] for 2 days at 35°C in a humidified chamber in ambient atmosphere.
[4]The Chicken Embryo Infectious Dose 50% ($CEID_{50}$) 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $CEID_{50}$ provides a measure of the infectious titer (or infectivity) of a virus preparation.
[5]48 hours at 35°C in a humidified chamber without CO_2.
[6]The primers are described in Hoffmann, E., et al. "Universal Primer Set for the Full-Length Amplification of All Influenza A Viruses." Arch. Virol. 146 (2001): 2275-2289. PubMed: 11811679.
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 17 JUL 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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