

Kilbourne F9: A/New Jersey/11/76 (H1N1)

Catalog No. NR-3595

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A Virus, A/New Jersey/11/76 (H1N1), (Kilbourne F9).

Lot^{2,3}: 58404374

Manufacturing Date: 07NOV2008

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs¹ Hemagglutination (HA) assay using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species-Specific Region (~440 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID₅₀ Assay^{4,5} in Embryonated Chicken Eggs¹	Report results	8.9 X 10 ⁷ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA⁶	~ 470 bp amplicon	~ 470 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 Sterility (BacT/ALERT[®] 3D Microbial Detection System) 14-day incubation of NR-3595: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	No growth No growth No growth No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth 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.

²Derived from NIAID Catalog No. V-331-0E5448.

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.

⁵48 hours at 35°C in a humidified chamber.

⁶The primers are described in Lee, M.-S., et al. "Identification and Subtyping of Avian Influenza Viruses by Reverse Transcription-PCR." *J. Virol. Methods* 97 (2001): 13-22. PubMed: 11483213.

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

Date: 06 JAN 2009

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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