

Kilbourne F74: A/turkey/Massachusetts/3740/75 (HA) x A/Beijing/353/89 (NA) x A/Puerto Rico/8/34 (H6N2), Reassortant X-110

Catalog No. NR-3516

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/353/89 (NA) x A/Puerto Rico/8/34 (H6N2) (Kilbourne F74; X-110).

Lot^{2,3}: 58885098

Manufacturing Date: 23OCT2009

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs¹ Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species-Specific Region Matrix (M) gene (695 nucleotides) Hemagglutinin (HA) gene (297 nucleotides)	Influenza A virus Influenza A virus	Influenza A virus Influenza A virus
Titer by CEID₅₀ Assay^{4,5} in Embryonated Chicken Eggs¹	Report results	2.8 X 10 ⁹ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA M gene ⁶ HA gene, subtype 6 (H6) ⁷	~ 1030 bp amplicon ~ 302 bp amplicon	~ 1030 bp amplicon ~ 302 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 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 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-331-0E5019

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 48 hours 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 M gene 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.

⁷The H6 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.

⁸Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 21 JUN 2010

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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