

Kilbourne F109: A/equine/Prague/1/56 (HA) x A/Aichi/2/68 (NA) x A/Puerto Rico/8/34 (H7N2), Reassortant X-32

Catalog No. NR-3535

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A Virus, A/equine/Prague/1/56 (HA) x A/Aichi/2/68 (NA) x A/Puerto Rico/8/34 (H7N2) (Kilbourne F109; X-32).

Lot^{2,3}: 58133747

Manufacturing Date: 18APR2008

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 ⁴	Positive	Positive
Sequencing of Species-Specific Region (~ 680 nucleotides)	Influenza A virus	Influenza A virus
Titer by CEID₅₀ Assay^{5,6} in Embryonated Chicken Eggs¹	Report results	2.8 X 10 ⁹ CEID ₅₀ /mL
RT-PCR Assay of Extracted RNA⁷	~ 1030 bp amplicon	~ 1030 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-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania.

²Derived from NIAID Catalog No. V-331-0E5148.

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber without CO₂.

⁴HA assay was performed at 4°C. This reassortant has greater neuraminidase (NA) activity than HA activity and more NA per virion. Therefore, the HA activity cannot be measured at room temperature because of rapid elution of the virus from red blood cells.

⁵The 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.

⁶48 hours at 35°C in a humidified chamber without CO₂.

⁷The 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.

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

Date: 25 SEP 2008

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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