

Kilbourne F8: A/New Jersey/11/76 (H1N1) Mutant, Low (L) Yield

Catalog No. NR-3477

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with a low (L) yield mutant (Kilbourne F8) of influenza A virus, A/New Jersey/11/76 (H1N1).

Lot^{2,3}: 58432918

Manufacturing Date: 12DEC2008

| 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 Hemagglutinin gene (~ 410 nucleotides) | Influenza A virus | Influenza A virus |
| Titer by CEID₅₀ Assay^{4,5} in Embryonated Chicken Eggs¹ | Report results | 2.8 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 | 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-331-0E4372

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.

⁵2 days at 35°C in a humidified chamber

⁶The H1 gene 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: 25 FEB 2009

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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