

Kilbourne F87: A/turkey/Massachusetts/3740/1975 (HA) x A/Johannesburg/33/1994 (NA) x A/Puerto Rico/8/1934 (H6N2), Reassortant X-124E

Catalog No. NR-3586

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/turkey/Massachusetts/3740/1975 (HA) x A/Johannesburg/33/1994 (NA) x A/Puerto Rico/8/1934 (H6N2)

Lot^{2,3}: 61230171

Manufacturing Date: 10OCT2012

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 Hemagglutinin, Matrix and Neuraminidase Coding Regions Hemagglutinin (283 nucleotides) Matrix (921 nucleotides) Neuraminidase (470 nucleotides)	Consistent with A/turkey/Massachusetts/3740/1965 (H6N2) ⁴ Consistent with A/Puerto Rico/8/1934 (H1N1) Consistent with A/Johannesburg/33/1994 (H3N2)	99% identity with A/turkey/Massachusetts/3740/1965 (H6N2) (GenBank: CY087752) ⁴ 100% identity with A/Puerto Rico/8/1934 (H1N1) (Gen Bank: CY105897) 99% identity with A/Johannesburg/33/1994 (H3N2) (GenBank: CY121343)
Titer by CEID₅₀ Assay^{5,6} in Embryonated Chicken Eggs¹	Report results	1.1 × 10 ⁸ CEID ₅₀ per mL
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 Blood agar, 37°C, aerobic 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

¹9- to 10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

²Derived from NIAID Catalog No. V-331-0E5429

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber

⁴There is no sequence information for influenza A virus, A/turkey/Massachusetts/3740/1975 (H6N2) in the NCBI database or any of the various influenza databases. The hemagglutinin gene sequence obtained for NR-3586 is 99% identical to the published sequence of influenza A virus, A/turkey/Massachusetts/3740/1965 (H6N2). A review of the literature suggests that these two strain designations likely represent the same virus isolate, and that the progenitor of reassortant X-124E was originally isolated in 1965.

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.

⁶2 days at 35°C in a humidified chamber

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

Certificate of Analysis for NR-3586

Date: 23 JUL 2013

Signature: *Michael R. Gynther*

Title: Technical Manager, BEI Authentication or designee

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