

Certificate of Analysis for NR-3697

Kilbourne F45: B/Massachusetts/1/1971 (HA) x B/Lee/1940 (NA)

Catalog No. NR-3697

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza B virus, B/Massachusetts/1/1971 (HA) x B/Lee/1940 (NA)

Lot^{2,3}: 61995655 Manufacturing Date: 30JUL2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Neuramindase Coding Regions Hemagglutinin (988 nucleotides) Neuraminidase (575 nucleotides)	Consistent with influenza B virus ⁴ Consistent with B/Lee/1940	Consistent with influenza B virus ⁴ 100% identity with B/Lee/1940 (Gen Bank: CY115113)
Titer by CEID ₅₀ Assay ^{5,6} in Embryonated Chicken Eggs ¹	Report results	5.0×10^7 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
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 Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 31 OCT 2013 Signature: Mishael Q. Gypla

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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²Derived from NIAID Catalog No. V-331-0E5473B

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 33°C in a humidified chamber

⁴There is no sequence information for influenza B virus, B/Massachusetts/1/1971 in the NCBI database. The hemagglutinin gene sequence obtained for NR-3697 at BEI Resources has 98% to 99% identity with several contemporary influenza B virus isolates.

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 33°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.