

Certificate of Analysis for NR-3549

Kilbourne F95: A/Shenzhen/227/1995 (HA, NA) x A/Puerto Rico/8/1934 (H1N1),

Reassortant X-133

Catalog No. NR-3549

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with reassortant influenza A virus, A/Shenzhen/227/1995 (HA, NA) x A/Puerto Rico/8/1934 (H1N1)

Lot^{2,3}: 61416450 Manufacturing Date: 06DEC2012

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (458 nucleotides) Matrix (952 nucleotides)	Consistent with A/Shenzhen/227/1995 (H1N1) Consistent with A/Puerto Rico/8/1934 (H1N1)	99% identity with A/Shenzhen/227/1995 (H1N1) (GenBank: CY125052) 99% identity with A/Puerto Rico/8/1934 (H1N1) (Gen Bank: CY105897)
Titer by CEID ₅₀ Assay ^{4,5} in Embryonated Chicken Eggs ¹	Report results	8.9×10^9 CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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

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

Date: 16 APR 2013

Signature: Milhard Q. Com ha

Title: Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5231

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

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.