

Certificate of Analysis for NR-29028

Influenza A Virus, A/Puerto Rico/8-9VMC3/1934 (H1N1)¹

Catalog No. NR-29028

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs² infected with influenza A virus, A/Puerto Rico/8-9VMC3/1934 (H1N1)

Lot³: 60300876 Manufacturing Date: 30SEP2011

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ² Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Species- and Strain-Specific Regions Hemagglutinin (1016 nucleotides) Matrix gene (889 nucleotides)	Consistent with A/Puerto Rico/8-9VMC3/1934 (H1N1) (GenBank: CY083998) Consistent with A/Puerto Rico/8-9VMC3/1934 (H1N1) (GenBank: CY083999)	99% identity with A/Puerto Rico/8-9VMC3/1934 (H1N1) (GenBank: CY083998) ⁴ 99% identity with A/Puerto Rico/8-9VMC3/1934 (H1N1) (GenBank: CY083999)
Titer by CEID ₅₀ Assay ^{5,6} in Embryonated Chicken Eggs ²	Report results	8.9 × 10 ⁸ CEID ₅₀ /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

Deposited and labeled as influenza A virus, A/Puerto Rico/8-34-9VMC3/2010 (H1N1)

Date: 07 JUN 2012

Signature: Dorothy C. Young

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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²10- to 11-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

³Grown in the allantoic cavity of embryonated chicken eggs² for 2 days at 35°C in a humidified chamber

⁴Nucleotide sequencing of this region of the hemagglutinin gene of NR-29028 confirmed the presence of the point mutation responsible for the E156K substitution; there are two single nucleotide gaps in the HA2 region of the BEI Resources sequence as compared to CY083998.

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