

Certificate of Analysis for NR-45130

Influenza A Virus, A/red knot/Delaware Bay/240/1994 (H1N8)

Catalog No. NR-45130

Product Description: Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs¹ infected with influenza A virus, A/red knot/Delaware Bay/240/1994 (H1N8)

Lot²: 62558182 Manufacturing Date: 30APR2014

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 Matrix Coding Regions Hemagglutinin (439 nucleotides) Matrix (938 nucleotides)	Consistent with A/red knot/Delaware Bay/ 240/1994 (H1N8) Consistent with A/red knot/Delaware Bay/ 240/1994 (H1N8)	99% identity with A/red knot/Delaware Bay/ 240/1994 (H1N8) (GenBank: CY136856) 100% identity with A/red knot/Delaware Bay/ 240/1994 (H1N8) (GenBank: CY136857)
Titer by CEID ₅₀ Assay ^{3,4} in Embryonated Chicken Eggs ¹	Report results	$8.9 \times 10^9 \text{CEID}_{50} \text{per mL}$
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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: 17 DEC 2014 Signature: Michael R. Comple

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

³The 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.

⁴2 days at 35°C in a humidified chamber

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.