

Certificate of Analysis for NR-3176

Influenza A Virus, A/Wilson-Smith/1933 (H1N1)

Catalog No. NR-3176

This reagent is the property of the U.S. Government.

Product Description: Pooled allantoic fluid from embryonated chicken eggs infected with influenza A virus, A/Wilson-Smith/1933 (H1N1).

Lot: V-301-081-000 Manufacturing Date: 03DEC1962

TEST	SPECIFICATIONS	RESULTS (SEP 2011)
Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination assay	Positive	Positive
Sequencing of Hemagglutinin Gene (337 nucleotides)	Consistent with Influenza A/Wilson-Smith/1933 (H1N1) GenBank: CY009604	99% identity with Influenza A/Wilson-Smith (H1N1) GenBank: CY009604
Titer by CEID ₅₀ Assay ^{2,3} in Embryonated Chicken Eggs ¹	Report results	8.9 × 10 ⁷ CEID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁴ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep 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

¹⁰ to11-day-old SPF Embryonated Chicken Eggs.

Date: 27 FEB 2012

Signature: Dorothy C. Young

Title:

Technical Manager, BEI Authentication or designee

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

²The 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.

³2 days at 35°C in a humidified chamber without CO₂.

⁴Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.