Certificate of Analysis for NR-12283

Influenza A Virus, A/Brisbane/10/07 (H3N2)

Catalog No. NR-12283

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs[1] infected with influenza A virus, A/Brisbane/10/07 (H3N2).

Lot[2,3]: 58550258 **Manufacturing Date: 13MAR2009**

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 Species-Specific Region (880 nucleotides)	Influenza A virus	Influenza A virus
Titer by $CEID_{50}$ Assay[4,5] in Embryonated Chicken Eggs[1]	Report results	2.8 X 10^8 $CEID_{50}$/mL
RT-PCR Assay of Extracted RNA[6]	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (BacT/ALERT® 3D Microbial Detection System) 14-day incubation of NR-12283: i NST culture bottle, 32°C, anaerobic i AST culture bottle, 32°C, aerobic	 No growth No growth	 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

[1]10 to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania
[2]Source virus for this lot was prepared in embryonated chicken eggs and provided by the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH. Influenza A virus, A/Brisbane/10/07 (H3N2) was originally isolated in Brisbane, Australia in February 2007 from a human nasal swab. Following isolation this strain was passaged in eggs at the CDC followed by two egg passages at Baylor College of Medicine prior to deposition.
[3]Grown in the allantoic cavity of embryonated chicken eggs[1] for 48 hours at 35°C in a humidified chamber
[4]The Chicken Embryo Infectious Dose 50% ($CEID_{50}$) 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $CEID_{50}$ provides a measure of the infectious titer (or infectivity) of a virus preparation.
[5]2 days at 35°C in a humidified chamber
[6]The primers are described in Hoffmann, E., et al. "Universal Primer Set for the Full-Length Amplification of All Influenza A Viruses." Arch. Virol. 146 (2001): 2275-2289. PubMed: 11811679.

Date: 29 SEP 2009 **Signature:** Signature on File

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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