

## **Certificate of Analysis for NR-4229**

## Influenza A Virus, A/Denver/1/57 (H1N1)

Catalog No. NR-4229

(Derived from ATCC® VR-546™)

**Product Description:** Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with influenza A virus, A/Denver/1/57 (H1N1).

Lot<sup>2</sup>: 7694222 Manufacturing Date: 27OCT2006

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs <sup>1</sup> Hemagglutination assay	Active	Active
Sequencing of Species-Specific Region	Influenza A virus	Influenza A virus
Titer by CEID <sub>50</sub> Assay <sup>3,4</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	1.6 X 10 <sup>9</sup> CEID <sub>50</sub> /mL
RT-PCR Assay of Extracted RNA <sup>5</sup>	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth <sup>6</sup> , 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 <sub>2</sub>	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

<sup>&</sup>lt;sup>1</sup>10-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, PA.

**Date:** 25 SEP 2008 **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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<sup>&</sup>lt;sup>2</sup>NR-4<sup>2</sup>29 was produced by growth of ATCC<sup>®</sup> VR-546<sup>™</sup> (Lot 216486) in the allantoic cavity of embryonated chicken eggs<sup>1</sup> for 2 days at 35°C in a humidified chamber without CO<sub>2</sub>.

<sup>&</sup>lt;sup>3</sup>The Chicken Embryo Infectious Dose 50% (CEID<sub>50</sub>) 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the CEID<sub>50</sub> provides a measure of the infectious titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>4</sup>48 hours at 35°C in a humidified chamber without CO<sub>2</sub>.

<sup>&</sup>lt;sup>5</sup>The primers are described in Hoffmann, E., et al. "Universal Primer Set for the Full-Length Amplification of All Influenza A Viruses." <u>Arch. Virol.</u> 146 (2001): 2275-2289. PubMed: 11811679.

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