

## **Certificate of Analysis for NR-2758**

## Influenza A Virus, A/mallard duck/Pennsylvania/10218/84 (H5N2)

Catalog No. NR-2758

(Derived from ATCC® VR-1331™)

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with influenza A virus, A/mallard duck/Pennsylvania/10218/84 (H5N2).

Lot<sup>2</sup>: 7677042 Manufacturing Date: 29SEP2006

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>8</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

Date: 24 SEP 2007 Signature: Signature on file

> Title: Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>1</sup>10-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, PA.
<sup>2</sup>NR-2758 was produced by growth of ATCC<sup>®</sup> VR-1331™ (Lot 214340) in the allantoic cavity of embryonated chicken eggs<sup>1</sup> for 3 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% (LD50) 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>BM-M1 and BM-M-1027R primers; Obenauer, J. C., et al. "Large-Scale Sequence Analysis of Avian Influenza Isolates." <u>Science</u> 311 (2006): 1576– 1580. PubMed: 16439620.

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