

## **Certificate of Analysis for NR-9695**

## Influenza A Virus, A/Brisbane/10/2007 (HA, NA) x A/Puerto Rico/8/1934 (H3N2), Reassortant IVR-147

Catalog No. NR-9695

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with reassortant influenza A virus, A/Brisbane/10/2007 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)

Lot<sup>2,3</sup>: 58155274 Manufacturing Date: 09MAY2008

| TEST  | SPECIFICATIONS  | RESULTS   |
|---|---|---|
| Identification by Infectivity Using Embryonated Chicken Eggs <sup>1</sup> Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells   | Positive  | Positive  |
| Sequencing of Species-Specific Region (365 nucleotides)   | Influenza A virus   | Influenza A virus   |
| Titer by CEID <sub>50</sub> Assay <sup>4,5</sup> in Embryonated Chicken Eggs <sup>1</sup>   | Report results  | 8.9 × 10 <sup>7</sup> CEID <sub>50</sub> per mL                       |
| Sterility (21-day incubation)  Harpo's HTYE broth <sup>7</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<br>None detected  | None detected<br>None detected  |

<sup>&</sup>lt;sup>1</sup>11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 05 JUN 2013 Signature: Michael Q. 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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<sup>&</sup>lt;sup>2</sup>Source virus for this lot was prepared in embryonated hen eggs and provided by the Centers for Disease Control and Prevention, Atlanta, Georgia <sup>3</sup>Grown in the allantoic cavity of embryonated chicken eggs <sup>1</sup> for 2 days at 35°C in a humidified chamber

<sup>&</sup>lt;sup>4</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>5</sup>2 days at 35°C in a humidified chamber

<sup>&</sup>lt;sup>6</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>7</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.