

## Certificate of Analysis for NR-28621

## Influenza A Virus, A/Hong Kong/1/1968-1 Mouse-Adapted 12 (H3N2)

Catalog No. NR-28621

**Product Description:** Pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs<sup>1</sup> infected with influenza A virus, A/Hong Kong/1/1968-1 mouse-adapted 12 (H3N2)

**Passage History:** MKX/MK2E2/E1M2ML12M2E2/E2 (Isolating laboratory/WHO World Influenza Centre, London/Laboratory CDC, Health Canada, Ottawa/BEI); E# = Number passages in eggs; M# = Number passages in MDCK cells; MK# = Number passages in monkey kidney cells; ML# = Number passages in mouse lung; X = unknown

Lot<sup>2</sup>: 357 Manufacturing Date: 07DEC2016

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 Matrix Coding Region Matrix (843 nucleotides)	Consistent with A/Hong Kong/1- 1-MA-12/1968 (H3N2) <sup>3</sup>	99% identity with A/Hong Kong/1-1-MA-12/1968 (H3N2) (GenBank: CY033506) <sup>3</sup>
Strain Identification by Sequencing of PB2 Coding Region	Identity confirmed <sup>4</sup>	Identity confirmed <sup>4</sup>
Titer by CEID <sub>50</sub> Assay <sup>5,6</sup> in Embryonated Chicken Eggs <sup>1</sup>	Report results	$8.9 \times 10^9$ 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 Blood agar, 37°C, aerobic 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>10-</sup> to 11-day-old SPF Embryonated Chicken Eggs acquired from Charles River, Norwich, Connecticut

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<sup>&</sup>lt;sup>2</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>3</sup>NR-28621 was deposited to BEI Resources as A/Hong Kong/1/1968-1 mouse-adapted 12 (H3N2), but nucleotide sequence obtained from the same source material by the NIAID Influenza Genome Sequencing Consortium was deposited to NCBI and IRD as A/Hong Kong/1-1-MA-12/1968 (H3N2).

<sup>&</sup>lt;sup>4</sup>The PB2 gene (RNA segment 1) of influenza A/Hong Kong/1-1-MA-12/1968 (H3N2) (GenBank: CY033512) contains a G to A transition at nucleotide 1587 relative to the parental wild type A/Hong Kong/1/1968 (H3N2) virus. This point mutation is unique to this isolate and serves to confirm the identity of the strain.

<sup>&</sup>lt;sup>5</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>62</sup> days at 35°C in a humidified chamber

<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.



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**Date:** 18 APR 2017

Signature:

BEI Resources Authentication

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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