b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-28634

Influenza A Virus, A/Hong Kong/1/1968-2 Mouse-Adapted 21-2 (H3N2)

Catalog No. NR-28634

Product Description: Influenza A virus, A/Hong Kong/1/1968-2 mouse-adapted 21-2 (H3N2) was derived from a virus isolated from a human in Hong Kong in 1968. Vial contains pooled allantoic fluid from specific pathogen free (SPF) embryonated chicken eggs[1] infected with influenza A virus, A/Hong Kong/1/1968-2 mouse-adapted 21-2 (H3N2).

Passage History: MKX/MK2E2/E1M2E2M1E2ML21M2E2/E2 (Isolating laboratory/WHO World Influenza Centre, London/Laboratory CDC, Health Canada, Ottawa/BEI); E = Eggs; M = MDCK cells; MK = Monkey kidney cells; ML = Mouse lung; X = Unknown

Lot[2]: 70014486 **Manufacturing Date: 03MAY2018**

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 Matrix and Neuraminidase Coding Regions[3] Matrix (~ 750 nucleotides) Neuraminidase (~ 450 nucleotides)	 ≥ 98% identity with A/Hong Kong/1-2-MA21-2/1968 (H3N2) ≥ 98% identity with A/Hong Kong/1-2-MA21-2/1968 (H3N2)	 99.6% identity with A/Hong Kong/1-2-MA21-2/1968 (H3N2) (GenBank: CY033106.1) 99.7% identity with A/Hong Kong/1-2-MA21-2/1968 (H3N2) (GenBank: CY033107.1)
Strain Identification by Sequencing of Neuraminidase Coding Region[4]	Identity confirmed	Identity confirmed
Titer by $CEID_{50}$ Assay[5,6] in Embryonated Chicken Eggs[1]	Report results	8.9×10^7 $CEID_{50}$ per mL
Sterility (21-day incubation) Harpo's HTYE broth[7], 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_2	 No growth No growth No growth No growth No growth No growth No growth	 No growth No growth No growth 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]9- to11-day-old SPF Embryonated Chicken Eggs acquired from Charles River, Norwich, Connecticut, USA

[2]Grown in the allantoic cavity of embryonated chicken eggs[1] for 2 days at 35°C in a humidified chamber

[3]NR-28634 was deposited to BEI Resources as A/Hong Kong/1/1968-2 mouse-adapted 21-2 (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/1-2-MA21-2/1968 (H3N2).

[4]Among several specific mutations in the A/Hong Kong/1-2-MA21-2/1968 (H3N2) genome, the neuraminidase gene (RNA segment 6) (GenBank: CY033107) contains a G to A transition at nucleotide 1192 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.

[5]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.

[6]2 days at 35°C in a humidified chamber

[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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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