

Certificate of Analysis for NR-49064

Influenza A Virus, A/Brisbane/59/2007 (H1N1) (Mink Cell Adapted)

Catalog No. NR-49064

Product Description: Cell lysate and supernatant from *Neovison vison* lung epithelial cells¹ infected with mink cell adapted influenza A virus, A/Brisbane/59/2007 (H1N1)

Passage History: E2/E1/E2/E1C9Mv5 (Submission laboratory/CDC/Baylor College of Medicine/BEI Resources; E# = Number passages in eggs; C# = Number passages in MDCK cells; Mv# = Number passages in mink lung epithelial cells)

Lot²: 63013122 Manufacturing Date: 16JAN2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Mv 1 Lu Cells ¹ Hemagglutination assay using cell lysate and supernatant from infected MDCK cells and 0.5% chicken red blood cells Cytopathic effect	Positive Report results	Positive Cell enlargement, granularity, and detachment
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin gene (445 nucleotides) Matrix gene (933 nucleotides)	Consistent with A/Brisbane/59/2007 (H1N1) Consistent with A/Brisbane/59/2007 (H1N1)	99% identity with A/Brisbane/59/2007 (H1N1) (GenBank: CY163864) 99% identity with A/Brisbane/59/2007 (H1N1) (GenBank: CY163865)
Titer by TCID ₅₀ Assay ^{3,4,5} in MDCK Cells ¹	Report results	2.8 × 10 ⁵ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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 ₂	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

¹Mv 1 Lu cells: ATCC[®] CCL-64™

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

²Grown in Eagle's Minimal Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg per mL sodium bicarbonate (ATCC[®] 30-2003) supplemented with 0.125% bovine serum albumin (InvitrogenTM 15260-037) and 0.5 μg per mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma-Aldrich[®] T1426) for 2 days at 35°C with 5% CO₂.

³Determined by hemagglutination assay of culture supernatant using 0.5% chicken red blood cells.

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, 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 TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

⁵7 days at 35°C with 5% CO₂

⁶Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Date: 18 MAY 2015

Signature: Millard

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Fax: 703-365-2898