

Certificate of Analysis for NR-48598

Purified Influenza A Virus, A/Brisbane/59/2007 (H1N1) (Tissue Culture Adapted)

Catalog No. NR-48598

Product Description: Influenza A virus, A/Brisbane/59/2007 (H1N1) (tissue culture adapted) purified from infected Madin-Darby canine kidney (MDCK) cells¹ by high speed centrifugation of clarified supernatant

Passage History: E2/E1/E2/E1C10 (Submission laboratory/CDC/Baylor College of Medicine/BEI Resources; E# = Number passages in eggs; C# = Number passages in MDCK cells)

Lot^{2,3}: 62449041 Manufacturing Date: 20APR2015

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK Cells ¹	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species- and Strain-Specific Regions Hemagglutinin gene (448 nucleotides)	Consistent with A/Brisbane/59/2007 (H1N1)	100% identity with A/Brisbane/59/2007 (H1N1) (GenBank: CY163640)
Matrix gene (951 nucleotides)	Consistent with A/Brisbane/59/2007 (H1N1)	99% identity with A/Brisbane/59/2007 (H1N1) (GenBank: CY163633)
Titer by TCID ₅₀ Assay ^{4,5} in MDCK Cells ¹ with Indirect Fluorescent Antibody (IFA) Readout ⁶	Report results	1.6 × 10 ⁸ TCID 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 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

¹MDCK cells: ATCC® CCL-34™

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²Prepared by growth of influenza A virus, A/Brisbane/59/2007 (H1N1) (tissue culture adapted) (NR-31657, lot 62263122) in MDCK¹ cells followed by virus purification by high speed centrifugation of clarified supernatant

³Grown in Minimum Essential Medium containing Earle's salts (ATCC 30-2003) supplemented with 2 μg/mL TPCK-treated trypsin and 0.125% Bovine Serum Albumin (Gibco 15260-037) for 2 days at 35°C with 5% CO₂.

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.

⁵6 days at 35°C with 5% CO₂

⁶Using influenza A antibody FITC reagent (Millipore 5017)

⁷Atlas, 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: 21 JUL 2015

Signature: Milhard

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