

Certificate of Analysis for NR-3694

Kilbourne F61: A/WSN/1933 (HA, NA) x A/Rockefeller Institute/5/1957 (H1N1),

Reassortant NWS R7

Catalog No. NR-3694

Product Description: Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK) cells¹ infected with reassortant influenza A virus, A/WSN/1933 (HA, NA) x A/Rockefeller Institute/5/1957 (H1N1)

Lot^{2,3}: 61304790 Manufacturing Date: 18MAR2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK cells ¹	Report results	Cell rounding and clumping
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (449 nucleotides)	Consistent with A/WSN/1933 (H1N1)	100% identity with A/WSN/1933 (H1N1) (GenBank: CY034132)
Matrix (904 nucleotides)	Consistent with A/Rockefeller Institute/5/1957 (H2N2)	99% identity with A/Rockefeller Institute/5/1957 (H2N2) (Gen Bank: CY045845)
Titer by TCID ₅₀ Assay ^{4,5} in MDCK Cells ¹	Report results	1.6 × 10 ⁴ TCID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

¹MDCK; ATCC[®] CCL-34

Date: 03 JUN 2013 Signature: Minhael Q. Copy ha

Title: Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0TC476

³Grown in Dulbecco's Modified Eagle Medium (ATCC[®] 30-2002™) supplemented with 0.1% BSA (Invitrogen™ 15260-037) and 0.025 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (USB 22725) for 3 days at 35°C and 5% CO₂

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the infected cells, 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 infectious titer (or infectivity) of a virus preparation.

⁵⁶ days at 35°C and 5% CO2

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