

Certificate of Analysis for NR-3682

Kilbourne F64: A/WSN/1933 (HA, NA) x A/NWS/1934 x A/Rockefeller Institute/5/1957 (H1N1), Reassortant NWS-F (R12)

Catalog No. NR-3682

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/NWS/1934 x A/Rockefeller Institute/5/1957 (H1N1)

Lot^{2,3}: 61666201 Manufacturing Date: 20MAY2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK cells ¹	Report results	Cell rounding and clumping
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (444 nucleotides)	Consistent with A/WSN/1933 (H1N1)	100% identity with A/WSN/1933 (H1N1) (GenBank: CY034132)
Matrix (953 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	$8.9 \times 10^4 \text{TCID}_{50} \text{per mL}$
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 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
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; ATCC[®] CCL-34

Date: 22 AUG 2013 Signature: Michael Q. Complex

Title: Technical Manager, BEI Authentication or designee

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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

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

Fax: 703-365-2898

²Derived from NIAID Catalog No. V-331-0TC425

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.