

## **Certificate of Analysis for NR-3692**

Kilbourne F88: A/Nanchang/933/1995 (HA, NA) x A/Puerto Rico/8/1934 (H3N2), Reassortant X-125

Catalog No. NR-3692

**Product Description:** Cell lysate and supernatant from Madin-Darby Canine Kidney (MDCK) cells<sup>1</sup> infected with reassortant influenza A virus, A/Nanchang/933/1995 (HA, NA) x A/Puerto Rico/8/1934 (H3N2)

Lot<sup>2,3</sup>: 60341199 Manufacturing Date: 07MAR2012

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in MDCK Cells <sup>1</sup>	Cell rounding and sloughing	Cell rounding and sloughing
Sequencing of Hemagglutinin and Matrix Coding Regions Hemagglutinin (687 nucleotides)  Matrix (848 nucleotides)	Consistent with A/Nanchang/933/1995 (H3N2)  Consistent with A/Puerto Rico/8/1934 (H1N1)	99% Identity with A/Nanchang/933/1995 (H3N2) (Gen Bank: AF008725) 100% identity with A/Puerto Rico/8/1934 (H1N1)
45, 1	,	(GenBank: CY033578)
Titer by TCID <sub>50</sub> Assay <sup>4,5</sup> in MDCK Cells <sup>1</sup>	Report results	$8.9 \times 10^6$ TCID <sub>50</sub> per mL
Sterility (21-day incubation)		
Harpo's HTYE broth <sup>6</sup> , 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 <sub>2</sub>	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

<sup>&</sup>lt;sup>1</sup>MDCK cells (ATCC<sup>®</sup> CCL-34<sup>™</sup>)

Date: 01 NOV 2012 Signature: Dorothy C. Young

**Title:** Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>Derived from NIAID Catalog No. V-331-0TC471

<sup>&</sup>lt;sup>3</sup>Grown in Dulbecco's Modified Eagle Medium (ATCC<sup>®</sup> 30-2002™) supplemented with 0.125% BSA (Invitrogen™ 15260-037) and 0.2 μg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (USB 22725) for 2 days at 35°C and 5% CO₂

The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>&</sup>lt;sup>5</sup>7 days at 35°C and 5% CO<sub>2</sub>

<sup>&</sup>lt;sup>6</sup>Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.