

Certificate of Analysis for NR-2759

Influenza A Virus, A/WS/33 (H1N1) (Tissue-culture adapted)

Catalog No. NR-2759

(Derived from ATCC® VR-1520™)

Product Description: Cell lysate and supernatant from Madin-Darby canine kidney (MDCK) cells¹ infected with influenza A virus, A/WS/33 (H1N1) (Tissue-culture adapted).

Lot^{2,3}: 7677043 Manufacturing Date: 10MAR2007

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using MDCK Cells¹ Hemagglutination assay using cell lysate and supernatant from infected MDCK cells and 0.5% chicken red blood cells	Hemagglutination activity	Hemagglutination activity
Infection of MDCK cells	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region	Consistent with influenza A virus	Consistent with influenza A virus
Titer by TCID ₅₀ Assay ^{4,5} in MDCK Cells ¹	Report results	7.4 X 10 ⁶ TCID ₅₀ /mL
RT-PCR Assay of Extracted RNA ⁶	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth ⁷ , 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
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep 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 cells: ATCC[®] CCL-34[™].

Date: 15 NOV 2007 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²The inoculum for NR-2759 was ATCC® VR-1520™ (Lot 4057803).

³Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370) supplemented with 1 µg/mL Trypsin, TPCK-treated (USB® 22725), 0.125% Bovine Serum Albumin (Cambrex™ 14-471F), 10 mM HEPES Buffer (Invitrogen™ 15630-080), 2 mM L-glutamine (Invitrogen™ 25030), and 1 mM sodium pyruvate (Invitrogen™ 11360) for 4 days at 34°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.

⁵3 days at 33°C with 5% CO₂.

⁶Bm-M-1 and Bm-M-1027R primers; Hoffman, E., et al. "Universal Primer Set for the Full-length Amplification of All Influenza A Viruses." <u>Arch. Virol.</u> 146 (2001): 2275–2289. PubMed: 11811679.

⁷Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.