

Certificate of Analysis for NR-3237

Human Parainfluenza Virus 4a, M-25

Catalog No. NR-3237

This reagent is the property of the U.S. Government.

Product Description: Basal Medium Eagle with 0.2% SV-5 rabbit antiserum harvested from cultures of rhesus monkey kidney cells infected with human parainfluenza virus 4a (HPIV-4a), M-25, with 10% sucrose gelatin added

Lot: V-324-001-010 Manufacturing Date: ~ 1969

TEST	SPECIFICATIONS	RESULTS (FEB 2012)
Infectivity in LLC-MK2 Cells by Direct Fluorescent Antibody Staining Readout ^{1,2}	Report results	Fluorescence observed
Sequencing of HPIV-3-Specific Sequence (806 nucleotides)	Report results	97% identity with HPIV-4a, M-25 (GenBank: AB543336)
Titer by TCID ₅₀ Assay in Vero Cells with Direct Fluorescent Antibody Staining Readout ¹⁻⁴	Report results	1.6 x 10 ⁴ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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 Test Article nucleic acid	None detected None detected	None detected None detected

¹LLC-MK2 Rhesus Monkey Kidney (ATCC® CCL-7.1™)

Date: 14 AUG 2012 Signature: Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

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²Using Light Diagnostics™ Parainfluenza 4 FITC Reagent (Millipore 5034)

³Grown in Eagle¹s Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate (ATCC[®] 30-2003) and 2% fetal bovine serum (ATCC[®] 30-2020) for 9 days at 36°C with 5% CO₂

⁴The Tissue Culture Infectious Dose 50% (TCID₅₀) 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.

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.