

Certificate of Analysis for NR-44231

Human Respiratory Syncytial Virus, A2001/3-12, Purified from HEp-2 Cells

Catalog No. NR-44231

Product Description:

Human respiratory syncytial virus (hRSV), A2001/3-12 was isolated from a nasal wash from an infant with hRSV bronchiolitis in Nashville, Tennessee, on March 12, 2001. NR-44231 lot 70057940 was produced by infecting human epithelial carcinoma cells (HEp-2; ATCC® CCL-23™) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 6 days at 37°C with 5% CO₂. The virus was purified from clarified supernatant by high speed centrifugation.

Lot: 70057940 Manufacturing Date: 15FEB2023

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells	Syncytia, cell rounding and detachment	Syncytia, cell rounding and detachment
Sequencing of Species-Specific Region (~ 780 nucleotides)	≥ 98% identity with hRSV, A2001/3-12 (GenBank: JX069799)	98.2% identity with hRSV A2001/3-12 (GenBank: JX069799)
Titer by TCID₅ Assay in HEp-2 Cells by Fluorescent Antibody Testing¹,² (8 days at 37°C with 5% CO₂)	Report results	2.8 × 10 ⁵ TCID ₅₀ /mL
SDS-PAGE Analysis	Consistent with expected bands for hRSV	Consistent with expected bands for hRSV Four bands of ~90 kDa, ~50 kDa, ~45 kDa, ~34 kDa and ~28 kDa
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ³	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, aerobic	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

¹The 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. ²Using RSV DFA reagent (Millipore 5022)

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30 JUN 2023

Technical Manager or designee, ATCC Federal Solutions

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