

Certificate of Analysis for NR-28526

Human Respiratory Syncytial Virus, A2001/3-12

Catalog No. NR-28526

Product Description:

Human respiratory syncytial virus (RSV), A2001/3-12 was isolated from a nasal wash from an infant with RSV bronchiolitis in Nashville, Tennessee, USA, on March 12, 2001. NR-28526 lot 70039170 was produced by infecting *Homo sapiens* 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 4 days at 37°C with 5% CO₂.

Passage History:

HEp-2(12)/HEp-2(7) (Prior to deposit at BEI Resources/BEI Resources); HEp-2 = Homo sapiens carcinoma cells

Lot: 70039170 Manufacturing Date: 20OCT2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells	Cell rounding, syncytia formation and detachment	Cell rounding, syncytia formation and detachment
Identification by Direct Fluorescent Antibody (DFA) Assay¹	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~ 630 nucleotides)	≥ 98% identity with RSV, A2001/3-12 (GenBank: JX069799.1)	99.8% identity with RSV, A2001/3-12 (GenBank: JX069799.1)
Titer by TCID₅ Assay in HEp-2 Cells by DFA Readout ^{1,2} (8 days at 37°C with 5% CO₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Amplification of RSV Sequence by RT-PCR	~ 900 base pair amplicon	~ 900 base pair amplicon
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

¹Using Light Diagnostics™ Anti-Respiratory Syncytial Virus FITC Reagent (Millipore® 5022)

/Heather Couch/

Heather Couch 04 MAR 2021

Program Manager or designee, ATCC Federal Solutions

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²The 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.
³Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.