b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-28527

Human Respiratory Syncytial Virus, A1997/12-35

Catalog No. NR-28527

Product Description:

Human respiratory syncytial virus (RSV), A1997/12-35 was isolated from a nasal wash from an infant with RSV bronchiolitis in Nashville, Tennessee, USA, on December 22, 1997. NR-28527 lot 70039171 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 11 days at 37°C with 5% CO_2.

Passage History:

HEp-2(11)/HEp-2(14) (Prior to deposit at BEI Resources/BEI Resources); HEp-2 = *Homo sapiens* carcinoma cells

Lot: 70039171 **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) Assay[1]	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (700 nucleotides)	≥ 98% identity with RSV, A1997/12-35 (GenBank: JX069800.1)	100% identity with RSV, A1997/12-35 (GenBank: JX069800.1)
Titer by $TCID_{50}$ Assay in HEp-2 Cells by DFA Readout[1,2] (9 days at 37°C with 5% CO_2)	Report results	5.0×10^4 $TCID_{50}$ 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

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

[2]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

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

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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