

Certificate of Analysis for NR-52018

Recombinant Respiratory Syncytial Virus, A2 Expressing Green Fluorescent Protein (GFP) (rgRSV224)

Catalog No. NR-52018

Product Description:

Recombinant respiratory syncytial virus, A2 expressing green fluorescent protein (GFP) (rgRSV224) was developed using a historical strain of RSV, A2, originally isolated in the 1950s in the United States. NR-52018 lot 70059814 was produced by infecting *Homo sapiens* epithelial carcinoma cells (HEp-2; ATCC® CCL-23™) and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) containing Earle's Balanced Salt Solution with 25 mM HEPES, supplemented with 10% fetal bovine serum, (ATCC® 30-2020™) for 3 days at 37°C with 5% CO₂.

Passage History:

HEp-2(4)/Hep-2(1) (The Ohio State University/BEI Resources)

Lot: 70059814 Manufacturing Date: 12MAY2023

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEp-2 Cells	Syncytia formation and cell disruption	Syncytia formation and cell disruption
Confirmation of GFP Expression	Fluorescence observed	Fluorescence observed
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform	≥ 98% identity with RSV, A2 (GenBank: KT992094.1)	99.97% identity with RSV, A2 (GenBank: KT992094.1)
Titer by TCID₅ Assay in HEp-2 Cells by Cytopathic Effect¹ (5 days at 37°C with 5% CO₂)	Report results	1.6 × 10 ⁷ TCID ₅₀ /mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ²	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. ²Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Sonia Bjorum Brower/

Sonia Bjorum Brower
Technical Manager or designee, ATCC Federal Solutions

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BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898