Certificate of Analysis for NR-55284

Recombinant Vesicular Stomatitis Virus Expressing SARS-CoV-2 Spike (S) with Enhanced Green Fluorescent Protein (eGFP)

Catalog No. NR-55284

Product Description:

rVSV-SARS-CoV-2 S was generated by modification of a Vesicular Stomatitis Virus (VSV) antigenome to replace its native glycoprotein (G) with the full-length, wild-type severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Wuhan-Hu-1 Spike (S) gene (GenBank: MN908947.3). The antigenome also encodes for enhanced green fluorescent protein (eGFP). NR-55284 lot 70043361 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero; ATCC® CCL-81™) in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) and 1% Penicillin/Streptomycin solution (ATCC® 30-2300™) for 3 days at 37°C with 5% CO_2. Cell lysate and supernatant were clarified by centrifuging at 1240 × g for 15 minutes at 4°C.

Passage History:

V(7)/V(1) (Prior to deposit at BEI Resources/BEI Resources); V = Vero cells

Lot: 70043361 **Manufacturing Date: 09APR2021**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells	Syncytia and detachment	Syncytia and detachment
Identification by eGFP Expression in Vero Cells	Fluorescence observed	Fluorescence observed (Figure 1)
Genotypic Analysis by Next-Generation Sequencing	≥ 98% identity with Vesicular Stomatitis Indiana virus (GenBank: J02428.1) VSV G sequence absent SARS-CoV-2 S sequence confirmed	99.3% identity with Vesicular Stomatitis Indiana virus (GenBank: J02428.1) VSV G sequence absent SARS-CoV-2 S sequence confirmed[1]
Identification by eGFP Expression in Calu-3 Cells	Fluorescence observed	Fluorescence observed
Titer by $TCID_{50}$ Assay in Vero Cells by Cytopathic Effect and eGFP Expression[2] (5 days at 37°C and 5% CO_2)	Report results	8.9×10^5 $TCID_{50}$ per mL
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]rVSV-SARS-CoV-2 S is reported to have numerous point mutations within the SARS-CoV-2 S gene along with a 21 to 24 base pair deletion at the 3' end. The sequence obtained for NR-55284 is consistent with the depositor's sequence. For more information, please refer to Dieterle, M. E., et al. "A Replication-Competent Vesicular Stomatitis Virus for Studies of SARS-CoV-2 Spike-Mediated Cell Entry and Its Inhibition." Cell Host Microbe 23 (2020): 486-496. PubMed: 32738193.

[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.

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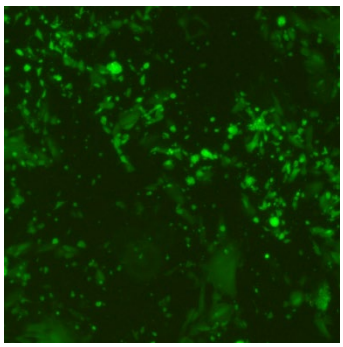
Figure 1: Identification by Infectivity in Vero Cells by GFP Expression



/Heather Couch/
Heather Couch 20 SEP 2021
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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