Certificate of Analysis for NR-53819

SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike D614G-Pseudotyped Lentivirus, Luc2/ZsGreen

Catalog No. NR-53819

Product Description:

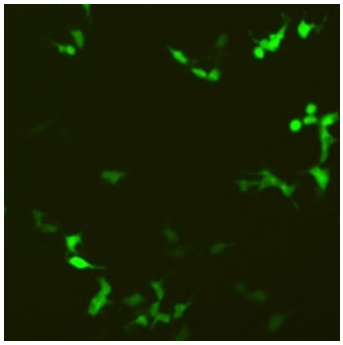
A pseudotyped lentivirus from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC_045512) was produced by transfection of purified plasmids (from BEI Resources NRC-52516, NRC-52517, NRC-52518, NRC-52519 and NRC-53765) in human embryonic kidney HEK293T cells (ATCC® CRL-3216) and grown for 2 days at 37°C in an aerobic atmosphere with 5% CO_2. The supernatant was harvested, filtered with a 0.45 µm filter and purified by 20% sucrose cushion. The lentiviral particles were resuspended in DMEM supplemented with 10% heat-inactivated fetal bovine serum. NR-53819 expresses a C-terminally truncated S glycoprotein containing a D614G mutation. The mutation increases titers of viral particles pseudotyped with SARS-CoV-2 S glycoprotein, as well as synthetic firefly luciferase (Luc2) and synthetic *Zoanthus* sp. green fluorescent protein (ZsGreen1).

Lot: 70042785 **Manufacturing Date: 01APR2021**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEK293-hACE2 Cells (BEI Resources NR-52511)	GFP expression	GFP expression (Figure 1)
Titer by $TCID_{50}$ Assay in HEK293-hACE2 Cells by Luciferase Assay[1,2] (2 days at 37°C with 5% CO_2)	> 10^5 relative luciferase units	1.7 × 10^6 relative luciferase units
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]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.
[2]0.1 µL NR-53819 per well
[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: GFP Expression



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

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