Product Information Sheet for NR-53818

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

Catalog No. NR-53818

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
A pseudotyped lentiviral form of the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC_045512) was produced in human embryonic kidney HEK293 cells and purified by sucrose cushion. NR-53818 expresses a C-terminally truncated S glycoprotein, which 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).[1,2] NR-53818 is produced from a plasmid kit (BEI Resources NR-52516, NR-52517, NR-52518, NR-52519 and NR-53742; kit NR-53816). Protocols for the use of these items are published, and updates are available at protocols.io.[1,3]

The S glycoprotein mediates viral binding to the host angiotensin converting enzyme 2 (ACE2). This protein forms a trimer, and when bound to a host receptor, allows fusion of the viral and cellular membranes. The S protein is a target for neutralizing antibodies.[4]

See Appendix I for assay information. Successful use of this product necessarily depends on third party reagents. It is critically important that users confirm effective assay performance in their own laboratories using the appropriate control templates.

Material Provided:
Each vial contains approximately 25 µL of purified lentiviral particles from transfected HEK293 supernatant in DMEM supplemented with 10% heat-inactivated fetal bovine serum. NR-53818 includes sufficient material for one 96 well plate neutralization assay.

Packaging/Storage:
NR-53818 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentivirus, Luc2/ZsGreen, NR-53818."

Biosafety Level: 2
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:
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References:
1. Bloom, J. and A. Balasz, Personal Communication.
2. Crawford, K. H. D., et al. "Dynamics of Neutralizing Antibody Titers in the Months after SARS-CoV-2 Infection." J. Infect. Dis. (2020): *in press*. PubMed: 33000143.

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3. Crawford, K. H. D., et al. "Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays." Viruses 12 (2020): E513. PubMed: 32384820.
4. Hulswit, R. J. G., C. A. M. de Haan and B. -J. Bosch. "Coronavirus Spike Protein and Tropism Changes." Adv. Virus Res. 96 (2016): 29-57. PubMed: 27712627.

Support Provided by NIAID

APPENDIX I: NEUTRALIZATION ASSAY

Reagents/Equipment

Reagent/Equipment	Source	Catalog Number
HEK-293T-hACE2 Cell Line	BEI Resources	NR-52511
Polyclonal Anti-SARS-Related Coronavirus 2 Spike Glycoprotein (IgG, Rabbit)	BEI Resources	NR-52947
Polybrene	Sigma	TR1003-G
Lumitrac Plate	Greiner	655075

Note: Recommended maximum antibody dilution is 5 µg/mL per well, with 3-fold dilutions. Black walled, clear bottom or white-walled 96 well plates can be used for luciferase assays.

1. Plate HEK293T-hACE2 cells at a density of 1.5 x 10^4 cells per well in a 96 well plate.
2. In a separate U-bottom 96 well plate prepare antibody dilutions in cell culture medium (DMEM supplemented with 10% FBS) in a final volume of 30 µL.
3. Prepare control wells
    a. Negative control (Mock)- add 50 µL cell culture medium
    b. Positive control (Spike-LP)- add 30 µL cell culture medium + 20 µL working solution (step 4)
4. Add DMEM supplemented with 10% FBS to NR-53818 vial for a final volume of 2 mL (working solution); Dispense 20 µL working solution per well with antibody. Incubate plate 1 hour at 37°C under slow agitation.
5. Gently remove medium from cells; Add 10 µL of 30 µg/mL polybrene per well.
6. Inoculate cells with 50 µL Spike-LP/antibody mix from U-bottom plate per well; Incubate cells at 37°C with 5% CO_2 for 1 to 3 hours. Add 90 µL cell culture medium and incubate for 48 hours at 37°C with 5% CO_2.
7. Perform luciferase assay or analyze GFP by microscopy.