SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike D614G-Pseudotyped Lentiviral Kit

Catalog No. NR-53817

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-53817 is intended for producing pseudotyped particles/pseudovirions and is not for soluble protein expression.

The severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC_045512) spike-pseudotyped lentiviral kit (NR-53817) is designed to generate pseudotyped lentiviral particles with the spike (S) glycoprotein gene, as well as luciferase (Luc2) and green fluorescent protein (GFP). Protocols for the use of these items are published, and updates are available at protocols.io.[1,2]

NR-53817 consists of the five plasmids listed in Table 1. Descriptions of each component are included below.

Table 1: SARS-CoV-2 Lentiviral Kit

Plasmid Type	Insert	BEI Resources Catalog Number
Viral Entry Protein	S D614G Glycoprotein	NR-53765
Lentiviral Backbone	Luc2; ZsGreen	NR-52516
Helper Plasmid	Gag; pol	NR-52517
Helper Plasmid	Tat1b	NR-52518
Helper Plasmid	Rev1b	NR-52519

NR-53765 was designed by codon optimizing the S glycoprotein sequence (residues 1 to 1252) for mammalian expression with a D614G mutation and deletion of the C-terminal 21 amino acids and subcloned into the pHDM vector under the cytomegalovirus (CMV) promoter.[1,2] Note: The vial label indicates this product has a 21 base pair deletion, but it is a 21 amino acid deletion.

NR-52516 was designed by fusing the synthetic firefly luciferase (Luc2) gene to the encephalomyocarditis internal ribosomal entry site (IRES) and synthetic *Zoanthus* sp. green fluorescent protein (ZsGreen1) gene, allowing simultaneous expression of Luc2 and the ZsGreen1 gene, which were subcloned into the pHAGE lentiviral backbone vector under the CMV promoter.[1,2,3,4] The Luc2 gene has been codon optimized for mammalian expression and has had cryptic transcription factor binding sites removed. The ZsGreen1 gene has been codon optimized for mammalian expression and engineered for brighter fluorescence.[3] In addition, the pHAGE vector includes the Woodchuck hepatitis virus post-transcriptional regulatory element to enhance levels of transcription and gene expression. The resulting size of the plasmid is approximately 9370 base pairs.

NR-52517 and NR-52518 were designed by codon optimizing the genes *gag* and *pol* and the gene *tat1b,* respectively, from the human immunodeficiency virus (HIV) and subcloning them into the pHDM vector under the CMV promoter.[1,2,4] The resulting plasmid sizes are approximately 8910 base pairs and 4830 base pairs, respectively.

NR-52519 was designed by codon optimizing the *rev1b* gene from HIV and subcloning into the pRC-CMV vector under the CMV promoter.[1,2,4] NR-52519 contains a neomycin (G418) selectable marker for mammalian expression. The resulting size of the plasmid is approximately 5900 base pairs.

NR-53765, NR-52516, NR-52517, NR-52518 and NR-52519 contain the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*.

The complete plasmid sequences and maps are provided on the BEI Resources webpage. The plasmids were produced in *E. coli* and extracted.

Material Provided:

Each kit contains one vial of each plasmid DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. The DNA concentrations and volumes provided are shown on the Certificate of Analysis. The vials should be centrifuged prior to opening. Note: The contents of each vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression.

Packaging/Storage:

NR-53817 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike D614G-Pseudotyped Lentiviral Kit, NR-53817."

Biosafety Level: 1

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.

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NR-53817 is claimed in U.S. Patent number 8,008,006 and European Patent number 1341808 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.

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References:

1. Bloom, J. and A. Balasz, Personal Communication.
2. 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.
3. Matz, M. V., et al. "Fluorescent Proteins from Nonbioluminescent *Anthozoa* Species." Nat. Biotechnol. 17 (1999): 969-973. PubMed: 10504696.
4. Murphy, G. J., et al. "Exogenous Control of Mammalian Gene Expression via Modulation of Translational Termination." Nat. Med. 12 (2006): 1093-1099. PubMed: 16892063.
5. 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.

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