Product Information Sheet for NR-53765

Vector pHDM Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene, D614G Mutant with C-Terminal Deletion

Catalog No. NR-53765

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
Note: The vial label indicates this product has a 21 base pair deletion but is a 21 amino acid deletion.

The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was designed by codon optimization of the S glycoprotein sequence (residues 1 to 1252) with a D614G mutation and deletion of the C-terminal 21 amino acids, and subcloned into the pHDM vector under the CMV promoter.[1] NR-53765 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. The resulting size of the plasmid is approximately 8310 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

NR-53765 is part of a lentiviral expression system, and additional BEI Resources items are required for successful expression. Lentiviral expression requires lentiviral helper plasmids (BEI Resources NR-52517, NR-52518 and NR-52519; kit NR-53817). Protocols for the use of these items are published.[2] The C-terminal truncation in NR-53765 increases titers of viral particles pseudotyped with SARS-CoV-2 spike.[3]

Note: NR-53765 does not include an antibiotic selection cassette for mammalian expression.

The S glycoprotein mediates viral binding to the host angiotensin converting enzyme 2 (ACE2). This protein forms a trimer, which when bound to a host receptor allows fusion of the viral and cellular membranes.[3] A key mutation in the S gene leading to a D614G substitution in the S glycoprotein has been identified in strains of European origin. This mutation is associated with increased viral loads in the upper respiratory tract, as well as increased virus neutralization by antibodies.[4,5,6,7]

Material Provided:
Each vial contains plasmid DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA concentration and volume provided are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening. Note: The contents of the vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression.

Packaging/Storage:
NR-53765 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: Vector pHDM Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene, D614G Mutant with C-Terminal Deletion, NR-53765."

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.

Disclaimers:
You are authorized to use this product for research use only. It is not intended for human use.

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References:

1. Bloom, J., 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.
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6. Zharko, D., et al. "The Spike D614G Mutation Increases SARS-CoV-2 Infection of Multiple Human Cell Types." bioRxiv (2020): doi.org/10.1101/2020.06.14.151357.
7. Plante, J. A., et al. "Spike Mutation D614G Alters SARS-CoV-2 Fitness." Nature (2020): *in press*. PubMed: 33106671.
8. Greaney, A. J., et al. "Comprehensive Mapping of Mutations to the SARS-CoV-2 Receptor-Binding Domain that Affect Recognition by Polyclonal Human Serum Antibodies." bioRxiv (2020): doi.org/10.1101/2020.12.31.425021.

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