Vector pCMV Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Ectodomain

Catalog No. NR-52421

For research use only. Not for human use.

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Manufacturer:
BEI Resources

Product Description:
Note: The label on the vial is incorrect; the correct vector is pCMV.[1,2] The pCMV vector is identical to the pcDNA3.1 vector used for other plasmids provided by Dr. Veesler.

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 optimizing the S glycoprotein ectodomain (residues 14 to 1211) for mammalian expression, fused to an N-terminal mu-phosphatase signal sequence and C-terminal trimerizing foldon domain and octa-histidine tag, and subcloned into the pCMV mammalian expression vector.[1,2] The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RRAR→SGAG; residues 682 to 685) and KV→PP mutations (residues 983 and 984). NR-52421 contains the beta-lactamase gene, TEM-116, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*, and a neomycin (G418) selectable marker for mammalian expression. The resulting size of the plasmid is approximately 9300 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

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.[3]

Material Provided:
Each vial contains 0.3 µg of plasmid DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. 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-52421 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 contributed by David Veesler for distribution through BEI Resources, NIAID, NIH: Vector pCMV Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Ectodomain, NR-52421. Work making use of this reagent should also cite Walls, A. C. and Y.-J. Park, et al. Cell 181 (2020): 281-292. PubMed: 32155444."

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Veesler, D., Personal Communication.
2. Walls, A. C., et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." Cell 181 (2020): 281-292. PubMed: 32155444.
3. 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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