

Modified pαH Vector Containing the Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein

Catalog No. NR-54980

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The vector for the spike (S) glycoprotein gene from middle east respiratory syndrome coronavirus (MERS-CoV), England 1 (GenBank: [NC_038294](#)) was designed by codon optimizing the full-length S sequence (residues 1 to 1291) for mammalian expression and subcloning into the pαH mammalian expression vector, which was modified by subcloning a T4 foldon trimerization domain, a HRV3C protease cleavage site and the octa-histidine and 2X Strep-tag® II tags downstream of the open reading frame.^{1,2} The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RVSR→ASVG; residues 748 to 751) and VL→PP mutations (residues 1060 and 1061). NR-54980 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli* (*E. coli*). NR-54980 is also referred to as VRC7578.¹ The resulting size of the plasmid is approximately 8000 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 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-54980 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: Modified pαH Vector Containing the Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein, NR-54980."

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/bmb15/index.htm.

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NR-54980 is claimed in U.S. Provisional Patent Application number 16/344774 and Global Patent Index publication number EP 3532095 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof. NR-54980 cannot be transferred to for-profit entities. For-profit entities wishing to obtain this material must inquire to NIAID's Technology Transfer and Intellectual Property Office with reference to NIH Ref. No. E-234-2016 by e-mailing jstein@mail.nih.gov and matthew.reiber@nih.gov. The Scripps Research Institute and Dartmouth College have rights to this material.

References:

1. Graham, B., Personal Communication.
2. Wrapp, D., et al. "Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation." *Science* 367 (2020): 1260-1263. PubMed: 32075877.
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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