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

Catalog No. NR-52563

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

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 sequence ectodomain (residues 1 to 1208) for mammalian expression and subcloning into the pαH mammalian expression vector.[1,2] The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RRAR→GSAS; residues 682 to 685) and KV→PP mutations (residues 986 and 987). The pαH vector was modified by subcloning an SV40 promoter upstream of the S gene insert, as well as subcloning a T4 foldon trimerization domain, HRV3C protease cleavage site, and the tags Twin-Strep-tag® (TST) and octa-histidine downstream of the S gene.[1,2,3] NR-52463 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. NR-52563 is also referred to as VRC7471.[1] The resulting size of the plasmid is approximately 8370 base pairs. The complete plasmid sequence and map (Figure 1) 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.[4]

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-52563 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 SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Ectodomain, NR-52563."

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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NR-52563 is claimed in U.S. Provisional Patent Application numbers 62/412,703 and 16/344,774 and the continuations,

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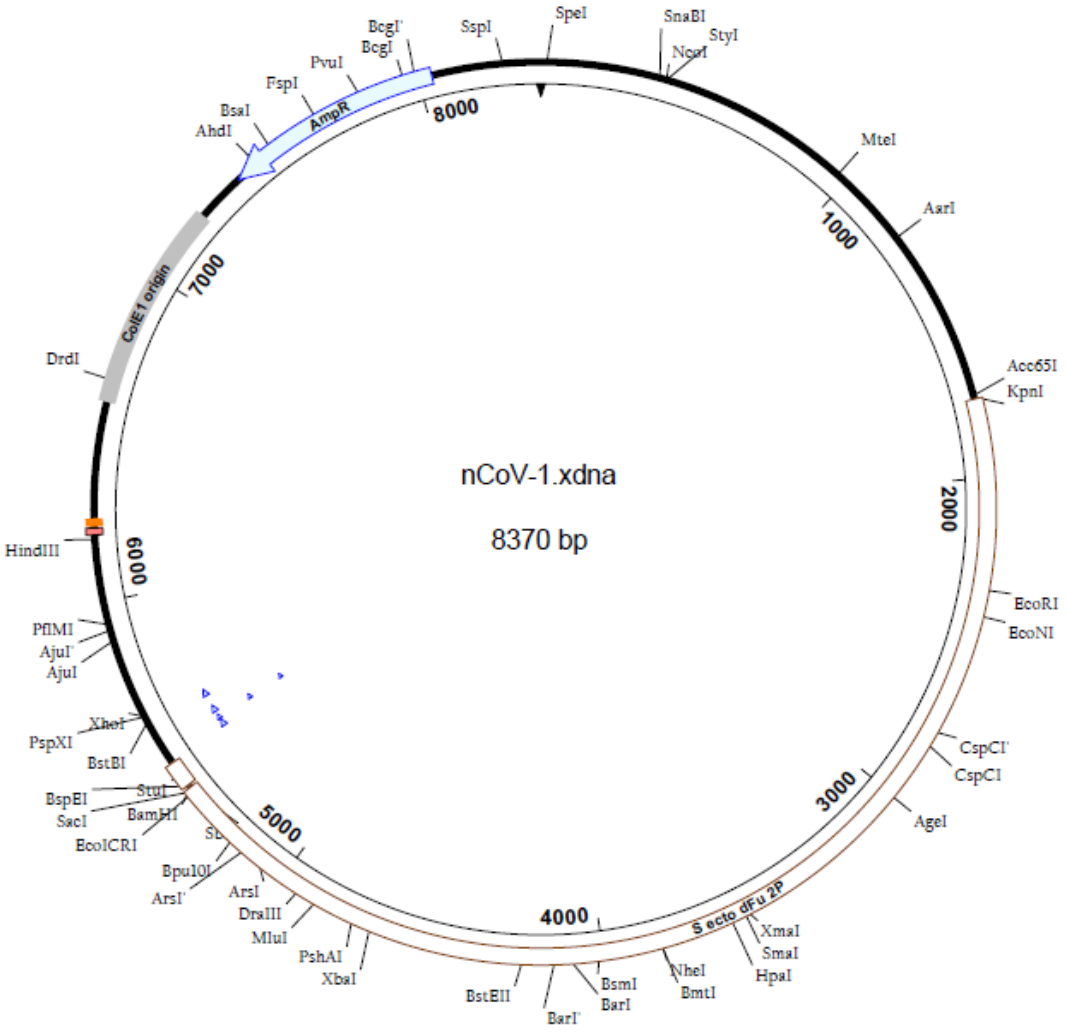
References:

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. Schmidt, T. G., et al. "Development of the Twin-Strep-tag® and its Application for Purification of Recombinant Proteins from Cell Culture Supernatants." Protein Expr. Purif. 92 (2013): 54-61. PubMed: 24012791.
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

Figure 1: Plasmid Map of NR-52563



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