Modified pCAGGS Vector Containing the SARS Coronavirus, Urbani Non-Structural Protein 3C Gene

Catalog No. NR-15204

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

The non-structural protein 3 (nsp3) gene from severe acute respiratory syndrome coronavirus (SARS-CoV), Urbani (GenBank: AY278741) was designed for expression of a C-terminal NSP3 fragment (residues 1319-1922; NSP3C) and cloned into the modified pCAGGS mammalian expression vector.[1,2] pCAGGS was modified by adding a hemagglutinin (HA) tag as well as a tobacco etch virus (TEV) cleavable BirA biotinylation tag C-terminal to the insert coding sequence. NR-15204 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

NSP3 is a multidomain protein located within the SARS-CoV ORF1ab polyprotein. The C-terminal fragment of NSP3 includes the ectodomain, Y1 and CoV-Y domains, although the exact function of this fragment is still under study.[3] Together with NSP4 and NSP6, NSP3 induces the formation of double-membrane vesicles, which are critical structures required for viral replication.[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-15204 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 pCAGGS Vector Containing the SARS Coronavirus, Urbani Non-Structural Protein 3C Gene, NR-15204."

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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References:

1. Kuhn, P., Personal Communication.
2. Smith, P. A., et al. "A Plasmid Expression System for Quantitative *in vivo* Biotinylation of Thioredoxin Fusion Proteins in *Escherichia coli*." Nucleic Acids Res. 26 (1998): 1414-1420. PubMed: 9490786.
3. Lei, J., Y. Kusov and R. Hilgenfeld. "NSP3 of Coronaviruses: Structures and Functions of a Large Multi-Domain Protein." Antiviral Res. 149 (2018): 58-74. PubMed: 29128390.

4. Angelini, M. M., et al. "Severe Acute Respiratory Syndrome Coronavirus Nonstructural Proteins 3, 4, and 6 Induce Double-Membrane Vesicles." mBio 4 (2013): e00524-13. PubMed: 23943763.

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