

Vector pCAGGS Containing SARS Coronavirus Nonstructural Protein 1

Catalog No. NR-15182

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

The nonstructural protein 1 (nsp1) coding sequence from the TOR-2 strain of the severe acute respiratory syndrome-related coronavirus (SARS-CoV) was subcloned into a modified pCAGGS mammalian expression vector.¹ The construct was engineered to encode a C-terminal fragment containing linker sequences, a hemagglutinin (HA) tag, a tobacco etch virus (TEV) protease cleavage site, and a 23 amino acid sequence that is site-specifically biotinylated by the *Escherichia coli* (*E.coli*) biotin ligase BirA.² The resulting plasmid was named pCC438. NR-15182 was produced in *E. coli* TOP10 cells (Invitrogen™) and extracted using a QIAGEN® Plasmid Plus Maxi Kit.

Nsp1 and associated cellular proteins can be efficiently purified from HEK293/ACE2 cells co-transfected with NR-15182 and a plasmid encoding BirA using streptavidin paramagnetic beads.^{1,2}

NR-15182 has been qualified for use in bacterial transformations.

Material Provided:

Each vial contains approximately 100 µL of plasmid DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.0). The DNA concentration and content are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening.

Packaging/Storage:

NR-15182 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 pCAGGS Containing SARS Coronavirus Nonstructural Protein 1, NR-15182.”

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. Cornillez-Ty, C.T., et al. “Severe Acute Respiratory Syndrome Coronavirus Nonstructural Protein 2 Interacts with a Host Protein Complex Involved in Mitochondrial Biogenesis and Intracellular Signaling.” J. Virol. 83 (2009): 10314-10318. PubMed: 19640993.
2. deBoer, E., et al. “Efficient Biotinylation and Single-Step Purification of Tagged Transcription Factors in Mammalian Cells and Transgenic Mice.” Proc. Natl. Acad. Sci. USA. 100 (2003): 7480-7485. PubMed: 12802011.

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