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SUPPORTING INFECTIOUS DISEASE RESEARCH

Vector paH Containing Respiratory Syncytial Virus A2 (RSV A2), Recombinant Glycoprotein F (+) FdTHS DS-Cav1 Gene (Prefusion)

Catalog No. NR-55425

This reagent is the tangible property of the U.S. Government.

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

NR-55425 is an expression vector encoding Respiratory Syncytial Virus A2 (RSV A2) recombinant prefusion F glycoprotein variant DS-Cav1. The construct consists of synthesized, mammalian codon-optimized RSV F(+) residues 1 to 513 [containing two sets of mutations: S155C AND S290C (DS) and S190F-V207L (Cav1)], a C-terminal T4 fibritin trimerization motif, thrombin cleavage site, hexa-histidine tag, and Strep-tag®II.^{1,2} RSV F(+) is derived from RSV A2 strain P03420) with three naturally occurring (GenPept: substitutions (P102A, I379V and M447V) for enhanced protein expression.² The vector backbone paH, is derived from mammalian expression vector pLEXm and contains the betalactamase gene, bla, to provide transformant selection through ampicillin resistance in Escherichia coli (E. coli).³ The insert is 1704 base pairs, and the resulting size of the plasmid is approximately 6250 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 conformational diversity of RSV F glycoprotein poses a major challenge in the design of effective subunit vaccines against RSV. Expression systems producing recombinant RSV F proteins in diverse conformational states are important tools to dissect the antibody response to natural RSV infection and following vaccination. The DS-Cav1 combination variant of RSV F glycoprotein was reported to form stable trimers and exhibited physical stability to extremes of temperature, pH, osmolality, and freeze-thaw.² NR-55425 can be used to elucidate binding and neutralizing antibody profiles in response to RSV.¹ It is often used in conjunction with vector paH eoncoding RSV A2 recombinant fusion glycoprotein dFP, (BEI Resources NR-55426).¹

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. <u>Note</u>: The contents of the vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression.

Packaging/Storage:

NR-55425 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 paH Containing Respiratory Syncytial Virus A2 (RSV A2), Recombinant Glycoprotein F (+) FdTHS DS-Cav1 Gene (Prefusion), NR-55425."

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. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

Disclaimers:

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NR-55425 is claimed in U.S. Patent Nos. 9738689 and 10017543 and the continuations, continuations in part, reissues and foreign counterparts thereof.^{4,5} NR-55425 cannot be transferred to for-profit entities.

References:

- 1. Graham, B., Personal Communication.
- McLellan, J. S. et al. "Structure-Based Design of a Fusion Glycoprotein Vaccine for Respiratory Syncytial Virus." <u>Science</u> 342 (2013): 592-598. PubMed: 24179220.
- Aricescu, A. R., et al. "A Time- and Cost-Efficient System for High-Level Protein Production in Mammalian Cells." <u>Acta Crystallogr. D. Biol. Crystallogr.</u> 62 (2006): 1243-1250. PubMed: 17001101.
- Kwong, P. D., et al. "Prefusion RSV F Proteins and their Use." <u>U.S. Patent No. 9738689</u>, 2017.
- Kwong, P. D., et al. "Prefusion RSV F Proteins and their Use." <u>U.S. Patent No. 10017543</u>, 2018.

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