

SARS-CoV Spike (S) Protein Δ TM, Recombinant from baculovirus

Catalog No. NR-722

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Contributor:

NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH.

Product Description:

NR-722 is a truncated, glycosylated recombinant form of the SARS-CoV spike (S) external envelope glycoprotein. A 60-amino acid deletion of the transmembrane domain (Δ TM) was made to the C-terminal end of the protein. A baculovirus signal sequence, MPLYKLLNVLWLVAVSNA, was added to the N-terminal end of the protein to enhance expression.¹ The SARS-CoV spike (S) protein Δ TM was produced in Sf9 insect cells using a baculovirus expression vector system^{2,3} and was purified using conventional chromatographic techniques. The baculovirus signal sequence is efficiently removed during translocation of the protein across the membrane. The resulting protein, NR-722, has a molecular weight of approximately 150,000 daltons. The predicted protein sequence of NR-722 is shown in Table 1 below.

Material Provided:

Each vial contains approximately 1 mg of NR-722 in 2.5 mM sodium phosphate (pH 7.2) and 150 mM NaCl.

Packaging/Storage:

NR-722 was packaged aseptically in glass serum vials. The product is provided on dry ice and should be stored at -20°C or colder immediately upon arrival. Repeated freeze-thaw cycles of this product should be avoided.

Note:

NR-722 may stick to glass when the protein is stored at low concentrations.

Functional Activity:^{2,3}

NR-722 was demonstrated to be functionally active based on its ability to bind to a recombinant form of its functional receptor, angiotensin I converting enzyme-2.⁴

Polyclonal antibody to the SARS-CoV S protein was shown to recognize NR-722 by Western blot analysis under both denaturing and non-denaturing conditions.¹ Monoclonal antibodies to the SARS-CoV S protein (BEI products NR-616, NR-617, NR-618 and NR-620) were shown to recognize SARS-CoV Spike Protein Δ TM (NR-722) by ELISA and by Western blot analysis when reducing agent was not present

in the SDS-PAGE gel. The monoclonal antibodies did not recognize the denatured protein by Western blot analysis when reducing agent was present in the SDS-PAGE gel.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: SARS-CoV Spike (S) Protein Δ TM, Recombinant from baculovirus, NR-722."

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, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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References:

1. Protein Sciences Corporation, Meriden, Connecticut, personal communication.
2. Smith, G. E., et al. Method for Producing Influenza Hemagglutinin Multivalent Vaccines Using Baculovirus. MG-PMC, LLC, assignee. U.S. Patent 5,762,939. 09 Jun. 1998.
3. Smith, G. E., et al. *Spodoptera frugiperda* Single Cell Suspension Cell Line in Serum-Free Media, Methods of Producing and Using. Protein Sciences Corporation, assignee. U.S. Patent 6,103,526. 15 Aug. 2000.
4. Li, W., et al. "Angiotensin-Converting Enzyme 2 is a Functional Receptor for the SARS Coronavirus." *Nature* 426 (2003): 450–454. PubMed: 14647384.

Table 1 – Predicted Protein Sequence

1	SDLDRCTTFD	DVQAPNYTQH	TSSMRGVYYP	DEIFRSDTLY	LTQDLFLPFY
51	SNVTGFHTIN	HTFGNPVIPF	KDGIYFAATE	KSNVVRGWVF	GSTMNKSQS
101	VIIINNSTNV	VIRACNFELC	DNPFFAVSKP	MGTQTHMIF	DNAFNCTFEY
151	ISDAFSLDVS	EKSGNFKHLR	EFVFKNKDGF	LYVYKGYQPI	DVVRDLPSPG
201	NTLKPFIKLP	LGINITNFRA	ILTAFSPAQD	IWGTSAAAYF	VGYLKPTTFM
251	LKYDENGIT	DAVDCSQNPL	AELKCSVKSF	EIDKGIYQTS	NFRVVPSPGD
301	VRFPNITNLC	PFGEVFNATK	FPSVYAWERK	KISNCVADYS	VLYNSTFFST
351	FKCYGVSATK	LNDLCFSNVY	ADSFVVKGDD	VRQIAPGQTG	VIADYNYKLP
401	DDFMGCVLAW	NTRNIDATST	GNVNYKYRYL	RHGKLRPFER	DISNVPFSPD
451	GKPCTPPALN	CYWPLNDYGF	YTTTGIGYQP	YRVVVLSEFL	LNAPATVCGP
501	KLSTDLIKQ	CVNFNFNGLT	GTGVLTPSSK	RFQPFQQFGR	DVSDFTDSVR
551	DPKTSEILDI	SPCSFGGVSV	ITPGTNASSE	VAVLYQDVNC	TDVSTAIHAD
601	QLTPAWRIYS	TGNNVFQTQA	GCLIGAEHVD	TSYECDIPIG	AGICASYHTV
651	SLLRSTSQKS	IVAYTMSLGA	DSSIAYSNNT	IAIPTNFSIS	ITTEVMPVSM
701	AKTSVDCNMY	ICGDSTECAN	LLLQYGSFCT	QLNRALSGIA	AEQDRNTREV
751	FAQVKQMYKT	PTLKYFGGFN	FSQILPDPLK	PTKRSFIEDL	LFNKVTLADA
801	GFMKQYGECL	GDINARDLIC	AQKFNGLTVL	PPLLTDDMIA	AYTAALVSGT
851	ATAGWTFGAG	AALQIPFAMQ	MAYRFNGIGV	TQNVLYENQK	QIANQFNKAI
901	SQIQESLTTT	STALGKLQDV	VNQNAQALNT	LVKQLSSNFG	AISSVLNDIL
951	SRLDKVEAEV	QIDRLITGRL	QSLQTYVTQQ	LIRAAEIRAS	ANLAATKMSE
1001	CVLGQSKRVD	FCGKGYHLMS	FPQAAPHGVV	FLHVTYVPSQ	ERNFTTAPAI
1051	CHEGKAYFPR	EGVFVFNGTS	WFITQRNFFS	PQIITTDNTF	VSGNCDVVIG
1101	IINNTVYDPL	QPELDSFKEE	LDKYFKNHTS	PDVDLGDISG	INASVVNIQK
1151	EIDRLNEVAK	NLNEGLIDLQ	ELGKYEQ		