Product Information Sheet for NR-52724

Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-Terminal Histidine and Twin-Strep® Tags, Recombinant from HEK293 Cells

Catalog No. NR-52724

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

For research use only. Not for human use.

Contributor and Manufacturer:

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Product Description:

A recombinant form of the spike (S) glycoprotein from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenPept: QHD43416) was produced by transient transfection of the plasmid VRC7471 into human embryonic kidney HEK293 cells, purified by immobilized metal affinity and StrepTactin™ Sepharose® chromatography, and dialyzed into buffer.[1,2] NR-52724 lacks the signal sequence and contains 1194 residues (ectodomain) of the SARS-CoV-2 S glycoprotein; the recombinant protein was stabilized by substitution at the furin S1/S2 cleavage site (RRAR→GSAS; residues 682 to 685) and KV→PP mutations (residues 986 and 987), and includes a T4 foldon trimerization domain, HRV3C protease cleavage site, and C-terminal octa-histidine and Twin-Strep® (TST) tags.[1,3] The predicted protein sequence is shown in Figure 1. NR-52724 has a theoretical molecular weight of 142,275 daltons. The crystal structure for trimeric S glycoprotein from SARS-CoV-2 has been solved at 3.46 Å resolution (PDB: 6VSB).[4]

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.[5]

Material Provided:

Each vial contains approximately 100 µL of NR-52724 in phosphate buffered saline (PBS; pH 7.0). The concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

Note: The long-term stability of this preparation is not known at this time. It is recommended that users confirm the activity of the product if not used within three months of receipt.

Packaging/Storage:

NR-52724 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -60°C or colder immediately upon arrival. Freeze-thaw cycles should be avoided.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein (Stabilized) from SARS-Related Coronavirus 2, Wuhan-Hu-1 with C-Terminal Histidine and Twin-Strep® Tags, Recombinant from HEK293 Cells, NR-52724."

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.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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NR-52724 was produced from the plasmid VRC7471 which is claimed in U.S. Provisional Patent Application numbers 62/412,703 and 16/344,774 and the continuations, continuations-in-part, re-issues and foreign counterparts

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References:

1. Magnani, D. M., Personal Communication.
2. Walls, A. C., et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." Cell 181 (2020): 281-292. PubMed: 32155444.
3. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." Nature 579 (2020): 265-269. PubMed: 32015508.
4. Wrapp, D., et al. "Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation." Science 367 (2020): 1260-1263. PubMed: 32075877.
5. 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 – Predicted Protein Sequence

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1    CVNLTTRTQL PPAYTNSFTR GVYYPDKVFR SSVLHSTQDL FLPFFSNVTW
51   FHAIHVSGTN GTKRFDNPVL PFNDGVYFAS TEKSNIIRGW IFGTTLDSKT
101  QSLLIVNNAT NVVIKVCEFQ FCNDPFLGVY YHKNNKSWME SEFRVYSSAN
151  NCTFEYVSQP FLMDLEGKQG NFKNLREFVF KNIDGYFKIY SKHTPINLVR
201  DLPQGFSALE PLVDLPIGIN ITRFQTLLAL HRSYLTPGDS SSGWTAGAAA
251  YYVGYLQPRT FLLKYNENGT ITDAVDCALD PLSETKCTLK SFTVEKGIYQ
301  TSNFRVQPTE SIVRFPNITN LCPFGEVFNA TRFASVYAWN RKRISNCVAD
351  YSVLYNSASF STFKCYGVSP TKLNDLCFTN VYADSFVIRG DEVRQIAPGQ
401  TGKIADYNYK LPDDFTGCVI AWNSNNLDSK VGGNYNYLYR LFRKSNLKPF
451  ERDISTEIYQ AGSTPCNGVE GFNCYFPLQS YGFQPTNGVG YQPYRVVVLS
501  FELLHAPATV CGPKKSTNLV KNKCVNFNFN GLTGTGVLTE SNKKFLPFQQ
551  FGRDIADTTD AVRDPQTLEI LDITPCSFGG VSVITPGTNT SNQVAVLYQD
601  VNCTEVPVAI HADQLTPTWR VYSTGSNVFQ TRAGCLIGAE HVNNSYECDI
651  PIGAGICASY QTQTNSPGSA SSVASQSIIA YTMSLGAENS VAYSNNSIAI
701  PTNFTISVTT EILPVSMTKT SVDCTMYICG DSTECSNLLL QYGSFCTQLN
751  RALTGIAVEQ DKNTQEVFAQ VKQIYKTPPI KDFGGFNFSQ ILPDPSKPSK
801  RSFIEDLLFN KVTLADAGFI KQYGDCLGDI AARDLICAQK FNGLTVLPPL
851  LTDEMIAQYT SALLAGTITS GWTFGAGAAL QIPFAMQMAY RFNGIGVTQN
901  VLYENQKLIA NQFNSAIGKI QDSLSSTASA LGKLQDVVNQ NAQALNTLVK
951  QLSSNFGAIS SVLNDILSRL DPPEAEVQID RLITGRLQSL QTYVTQQLIR
1001 AAEIRASANL AATKMSECVL GQSKRVDFCG KGYHLMSFPQ SAPHGVVFLH
1051 VTYVPAQEKN FTTAPAICHD GKAHFPREGV FVSNGTHWFV TQRNFYEPQI
1101 ITTDNTFVSG NCDVVIGIVN NTVYDPLQPE LDSFKEELDK YFKNHTSPDV
1151 DLGDISGINA SVVNIQKEID RLNEVAKNLN ESLIDLQELG KYEQGSGYIP
1201 EAPRDGQAYV RKDGEWVLLS TFLGRSLEVL FQGPGHHHHH HHHSAWSHPQ
1251 FEKGGGSGGG GSGGSAWSHP QFEK
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Spike ectodomain – **Residues 1 to 1194** (represents WT amino acid residues 15 to 1208)
RRAR to GSAS substitution of S1/S2 cleavage site – Residues 668 to 671
KV to PP stabilizing mutations – Residues 972 and 973
T4 foldon trimerization domain – Residues 1197 to 1223
HRV3C protease cleavage site – Residues 1227 to 1234
Octa-histidine tag – Residues 1236 to 1243
Twin-Strep® tag – Residues 1246 to 1274

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