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SUPPORTING INFECTIOUS DISEASE RESEARCH

Spike Glycoprotein (Stabilized) from MERS Coronavirus, England 1 with C-Terminal Histidine and Twin-Strep[®] Tags, Recombinant from HEK293 Cells

Catalog No. NR-53591

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

For research use only. Not for human use.

Contributor and Manufacturer:

D. Noah Sather, Associate Professor, Peter Myler and Jason McLellan, Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, Washington, USA

Product Description:

A recombinant form of the spike (S) glycoprotein from the England 1 strain (UK/H123990006/2012 isolate) of Middle East respiratory syndrome coronavirus (MERS-CoV; YP_007188579) was produced by transient GenPept: transfection into human embryonic kidney HEK293 cells and purified by immobilized metal affinity and gel filtration chromatography.^{1,2} NR-53591 lacks the signal sequence and contains 1274 residues (ectodomain) of the MERS-CoV S glycoprotein; the recombinant protein was stabilized by substitution at the furin S1/S2 cleavage site (RSVR→ASVG; residues 748 to 751) and VL→PP mutations (residues 1060 and 1061), and includes a T4 foldon trimerization domain, HRV3C protease cleavage site, and C-terminal octa-histidine and Twin-Strep[®] tags.^{1,2} The predicted protein sequence is shown in Figure 1. NR-53591 has a theoretical molecular weight of 148,650 daltons. The crystal structure for trimeric S glycoprotein from MERS-CoV has been solved at 4.00 Å resolution (PDB: 5W9P).²

The S glycoprotein mediates viral binding to the host dipeptidyl peptidase 4 (DPP4). 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.³

Material Provided:

Each vial contains approximately 100 μ L of NR-53591 in 10 mM HEPES, pH 7, 150 mM NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA). The concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

Packaging/Storage:

NR-53591 was packaged aseptically in cryovials. The product is provided on dry ice and should be stored at -20°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 MERS Coronavirus, England 1 with C-Terminal Histidine and Twin-Strep[®] Tags, Recombinant from HEK293 Cells, NR-53591."

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>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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NR-53591 is claimed in U.S. Provisional Patent Application number 16/344,774 and the continuations, continuations-inpart, re-issues and foreign counterparts thereof. The University of Texas-Austin, The Scripps Research Institute, Dartmouth College and the National Institutes of Health all have rights to this material.

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References:

- 1. Sather, D. N., P. Myler and J. McLellan, Personal Communication.
- Pallesen, J., et al. "Immunogenicity and Structures of a Rationally Designed Prefusion MERS-CoV Spike Antigen." <u>Proc. Natl. Acad. Sci. USA</u> 114 (2017): E7348-E7357. PubMed: 28807998.
- Hulswit, R. J. G., C. A. M. de Haan and B.-J. Bosch. "Coronavirus Spike Protein and Tropism Changes." <u>Adv.</u> <u>Virus Res.</u> 96 (2016): 29-57. PubMed: 27712627.

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Figure 1 – Predicted Protein Sequence

1	YVDVGPDSVK	SACIEVDIQQ	TFFDKTWPRP	IDVSKADGII	YPQGRTYSNI
51	TITYQGLFPY	QGDHGDMYVY	SAGHATGTTP	QKLFVANYSQ	DVKQFANGFV
101	VRIGAAANST	GTVIISPSTS	ATIRKIYPAF	MLGSSVGNFS	DGKMGRFFNH
151	TLVLLPDGCG	TLLRAFYCIL	EPRSGNHCPA	GNSYTSFATY	HTPATDCSDG
201	NYNRNASLNS	FKEYFNLRNC	TFMYTYNITE	DEILEWFGIT	QTAQGVHLFS
251	SRYVDLYGGN	MFQFATLPVY	DTIKYYSIIP	HSIRSIQSDR	KAWAAFYVYK
301	LQPLTFLLDF	SVDGYIRRAI	DCGFNDLSQL	HCSYESFDVE	SGVYSVSSFE
351	AKPSGSVVEQ	AEGVECDFSP	LLSGTPPQVY	NFKRLVFTNC	NYNLTKLLSL
401	FSVNDFTCSQ	ISPAAIASNC	YSSLILDYFS	YPLSMKSDLS	VSSAGPISQF
451	NYKQSFSNPT	CLILATVPHN	LTTITKPLKY	SYINKCSRFL	SDDRTEVPQL
501	VNANQYSPCV	SIVPSTVWED	GDYYRKQLSP	LEGGGWLVAS	GSTVAMTEQL
551	QMGFGITVQY	GTDTNSVCPK	LEFANDTKIA	SQLGNCVEYS	LYGVSGRGVF
601	QNCTAVGVRQ	QRFVYDAYQN	LVGYYSDDGN	YYCLRACVSV	PVSVIYDKET
651	KTHATLFGSV	ACEHISSTMS	QYSRSTRSML	KRRDSTYGPL	QTPVGCVLGL
701	VNSSLFVEDC	KLPLGQSLCA	LPDTPSTLTP	ASVGSVPGEM	RLASIAFNHP
751	IQVDQLNSSY	FKLSIPTNFS	FGVTQEYIQT	TIQKVTVDCK	QYVCNGFQKC
801	EQLLREYGQF	CSKINQALHG	ANLRQDDSVR	NLFASVKSSQ	SSPIIPGFGG
851	DFNLTLLEPV	SISTGSRSAR	SAIEDLLFDK	VTIADPGYMQ	GYDDCMQQGP
901	ASARDLICAQ	YVAGYKVLPP	LMDVNMEAAY	TSSLLGSIAG	VGWTAGLSSF
951	AAIPFAQSIF	YRLNGVGITQ	QVLSENQKLI	ANKFNQALGA	MQTGFTTTNE
1001	AFHKVQDAVN	NNAQALSKLA	SELSNTFGAI	SASIGDIIQR	LDPPEQDAQI
1051	DRLINGRLTT	LNAFVAQQLV	RSESAALSAQ	LAKDKVNECV	KAQSKRSGFC
1101	GQGTHIVSFV	VNAPNGLYFM	HVGYYPSNHI	EVVSAYGLCD	AANPTNCIAP
1151	VNGYFIKTNN	TRIVDEWSYT	GSSFYAPEPI	TSLNTKYVAP	QVTYQNISTN
1201	LPPPLLGNST	GIDFQDELDE	FFKNVSTSIP	NFGSLTQINT	TLLDLTYEML
1251	SLQQVVKALN	ESYIDLKELG	NYTY GSGYIP	EAPRDGQAYV	RKDGEWVLLS
1301	TFLGRSLEVL	FQGPG <u>HHHHH</u>	<u>HHH</u> SAWSHPQ	FEKGGGSGGG	GSGGSAWSHP
1351	QFEK				

Spike ectodomain - Residues 1 to 1274 (represents WT amino acid residues 18 to 1291)

RSVR to ASVG substitution of S1/S2 cleavage site - Residues 731 to 735

VL to PP stabilizing mutations – Residues 1043 and 1044 T4 foldon trimerization domain – Residues 1278 to 1303

HRV3C protease cleavage site - Residues 1307 to 1314

Octa-histidine tag – <u>Residues 1316 to 1323</u>

Twin-Strep® tag - Residues 1326 to 1354

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