



Daresbury Proteins

Product description

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Name: Recombinant SARS-CoV-2 Spike protein, D614G variant. Full-length soluble with foldon trimerization motif, mutated Furin recognition site and 2 stabilising mutations (K986P and V987P), based on/modified from Amanat *et al*, 2020.

Synonyms: Spike glycoprotein, S protein.

Species: Severe Acute Respiratory Syndrome Coronavirus 2

Source: HEK293

Amino Acids: 16-1213

Tag: 8xHis at the C terminus.

Predicted Molecular Weight: 137 kDa

Protein ID: P0DTC2

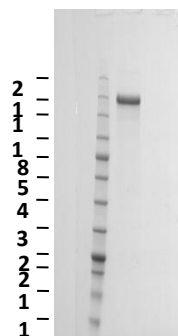
Sequence:

VNLTTRTQLP PAYTNSFTRG VYYPDKVFRS SVLHSTQDLF LPFFSNVTWF HAIHVSGTNG TKRFDNPVLP
 FNDGVYFAST EKSNIIRGWI FGTTLDSTQ SLLIVNNATN VVIKVCEFQF CNDPFLGVYY HKNNKSWMES
 EFRVYSSANN CTFEYVSQPF LMDLEGKQGN FKNLREFVFK NIDGYFKIYS KHTPINLVRD LPQGFSALEP
 LVDLPIGINI TRFQTLALH RSYLTPGDSS SGWTAGAAAY YVGYLQPRTF LLKYNENGTI TDAVDCALDP
 LSETKCTLKS FTVEKGIYQT SNFRVQPTES IVRFPNITNL CPFGEVFNAT RFASVYAWNR KRISNCVADY
 SVLYNSASFV TFKCYGVSPT KLNDLCFTNV YADSFVIRGD EVRQIAPGQT GKIADYNYKL PDDFTGCVIA
 WNSNNLDSKV GGNYNLYRL FRKSNLKPFE RDISTEIQY GSTPCNGVEG FNCYFPLQSY GFQPTNGVGY
 QPYRVVLSF ELLHAPATVC GPKKSTNLVK NKCVMFNFNNG LTGTGVLTES NKKFLPFQQF GRDIADTTDA
 VRDPQLEIL DITPCSFQGV SVITPGTNTS NQVAVLYQGV NCTEVPVAIH ADQLTPTRV YSTGSNVFQT
 RAGCLIGAEH VNNSYECDIP IGAGICASYQ TQTNSP**GGAS** VASQSI IAYT MSLGAENSV YSNNSIAIPT
 NFTISVTTEI LPVSMTKTSV DCTMYICGDS TECSNLLLQY GSFCTQLNRA LTGIAVEQDK NTQEVFAQVK
 QIYKTPPIKD FGGFNFSQIL PDPSKPSKRS FIEDLLFNKV TLADAGFIKQ YGDCLGDIAA RDLICAQKFN
 GLTVLPPLLT DEMIAQY TSA LLAGTITSGW TFGAGAALQI PFAMQMAYRF NGIGVTQNVL YENQKLIANQ
 FNSAIGKIQD SLSSTASALG KLQDVVNQNA QALNTLVKQL SSNFGAIVSS LNDILSRLD **P** **P**EAEVQIDRL
 ITGRLQSLQT YVTQQLIRAA EIRASANLAA TKMSECVLGQ SKRVDFCGKG YHLMSFPQSA PHGVVFLHVT
 YVPAQEKNT TAPAICHGDK AHFPREGV FV SNGTHWFVTQ RNFYEPQIIT TDNTFVSGNC DVVIGIVNNT
 VYDPLQPELD SFKEELDKYF KNHTSPDVLD GDISGINASV VNIQKEIDRL NEVAKNLNES LIDLQELGKY
 EQYIKWPS**GGG** **SGYIPEAPRD** **GQAYVRKDGE** **WVLLSTFLGS** **HHHHHHHH**

Product specifications

Estimated Molecular Weight, SDS-PAGE: ≈190 kDa

Grade & Purity: >90% as estimated by SDS-PAGE stained with Instant Blue Stain (Expedeon).



Daresbury Proteins Ltd. A company registered in England, UK. Company number 10835544.

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Endotoxins: Less than 0.1 ng/μg (1 IEU/μg), as measured by LAL method.

Formulation: PBS 20% Glycerol

Shipping

Product is shipped either on dry or wet ice or frozen gel packs. Upon receipt, store at -20°C to -70°C.

Product application and Storage

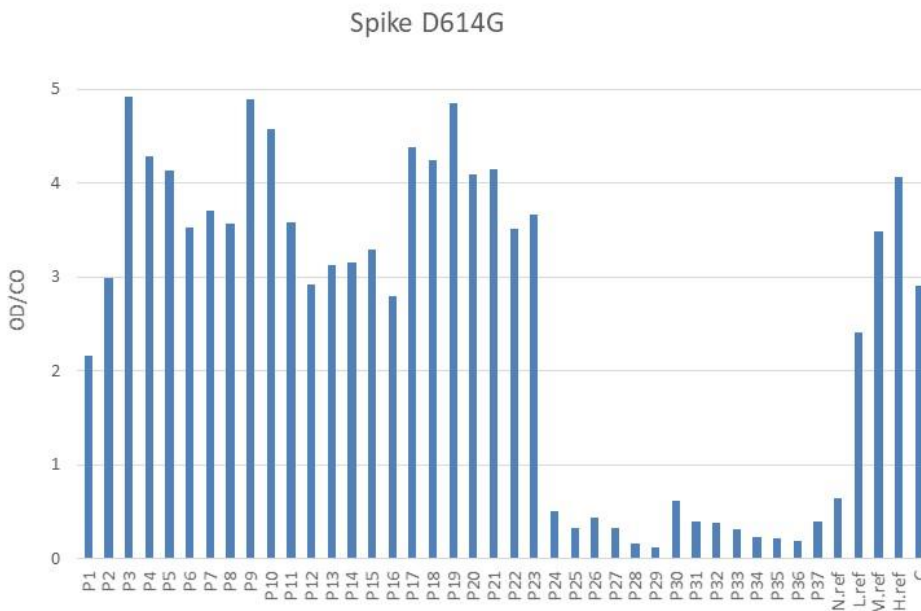
Storage: The protein should be stored at -20°C to -70°C preferably in small aliquots to avoid repeated freeze-thaw cycles.

Stability: At least 12 months at -20°C to -70°C and at least 1 month at 2°C to 8°C.

Application Note: For research purposes only. Not for use in humans.

Functional data

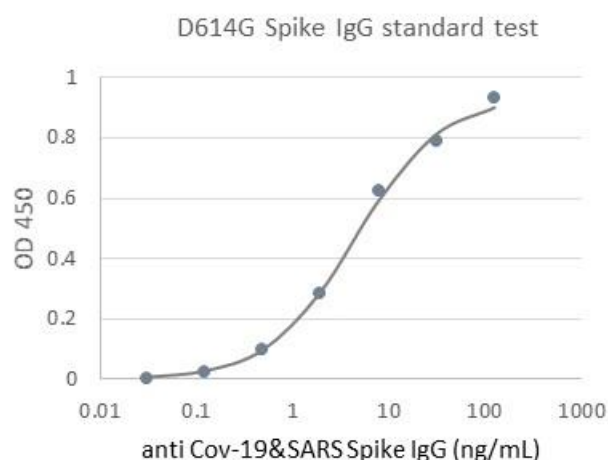
- Protein was immobilised on 96-well plates as a capture antigen. Human serum samples from a serological verification panel containing anti-SARS-CoV-2 antibodies from National Institute of Biological Standards and Controls, NIBSC (20/B770) were added to the plate for incubation and subsequent washing. Secondary human anti-IgG, HRP-conjugated, were added for detection in a sandwich ELISA format. The final colorimetric reaction occurs upon addition of TMB substrate which is quenched by the addition of acid solution.



NIBSC references (20/268):

- N – negative
- L – low positive
- M – medium positive
- H – high positive
- C – calibrant

2. Binding curve of anti-Cov-19&SARS S glycoprotein antibody IgG CR3022 (Absolute Antibody, Ab01680-10.0) to the trimeric Spike D614G:



Background Information

The first reports of atypical pneumonia in China were announced at the very end of 2019, in the city of Wuhan, Hubei province. Its cause was identified in January 2020 as a novel β -CoV (1), named SARS-CoV-2, which started spreading very quickly causing a global pandemic COVID-19.

Spike protein attaches the virion to the cell membrane by interacting with host receptor, initiating the infection. Binding to human ACE2 receptor and internalization of the virus into the endosomes of the host cell induces conformational changes in the Spike glycoprotein (2, 3). The protein forms a trimer *in vivo* mediating receptor binding and membrane fusion (4). It has been demonstrated by Florian Krammer's group that certain mutations and the inclusion of trimerization motif can stabilize recombinant Spike protein (5, 6).

The original Wuhan strain of the virus has become quickly replaced by its more transmissible variant, mainly determined by a single amino acid point mutation D614G (7).

References:

1. Zhu N., Zhang D., Wang W., et al. China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med.*, 2020;382:727-733.
2. Hoffmann M., Kleine-Weber H., Schroeder S., et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, 2020;181(2):271-280.
3. Wrapp D., Wang N., Corbett KS., et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 2020;367(6483):1260-1263.
4. Yin HS., Wen X., Paterson RG., Lamb RA., Jardetzky TS. Structure of the parainfluenza virus 5 F protein in its metastable, prefusion conformation. *Nature*, 2006;439(7072):38-44.
5. Amanat, F., Stadlbauer, D., Strohmeier, S., et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med.*, 2020;26:1033-1036.
6. Stadlbauer, D., Amanat, F., Chromikova, V., et al. SARS-CoV-2 seroconversion in humans: A detailed protocol for a serological assay, antigen production, and test setup. *Current Protocols in Microbiology*, 2020;57, e100.
7. Korber, B., W. Fischer, Gnanakaran, S., et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell*, 2020;182:1-16.

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