

Product Datasheet

Spike-RBD 319-541_HIS recombinant protein

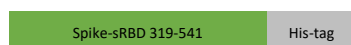
For research use only

Description:

Spike-RBD 319-541_HIS

Expressed in HEK-cell Expi293F system. Protein carries a poly-his tag at the N-terminus.

Correct sequence confirmed by Mass Spectrometry, where full coverage of the sequence has been obtained.



Calculated MW: 27 kDa

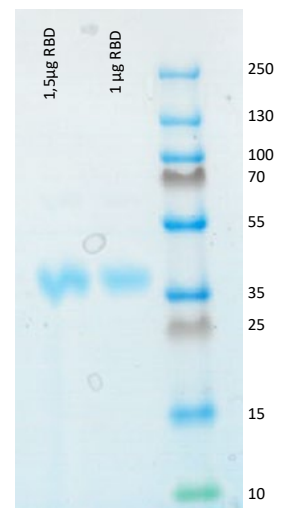
Protein migrates as approx. 37 kDa due to glycosylations (See SDS-page beside).

Glycan structures are confirmed, and glycosylation sites identified by Mass Spectrometry of protein samples with and without PNGaseF treatment. (see detailed results below).

Identified glycosylation sites: N42(IT) and N54(AT).

Glycan structures have a combined mass of approx. 6 kDa.

Dimerization percentage < 10%



Formulation: In PBS solution pH=7.4

Purification: Immobilized metal affinity chromatography, NiNTA.

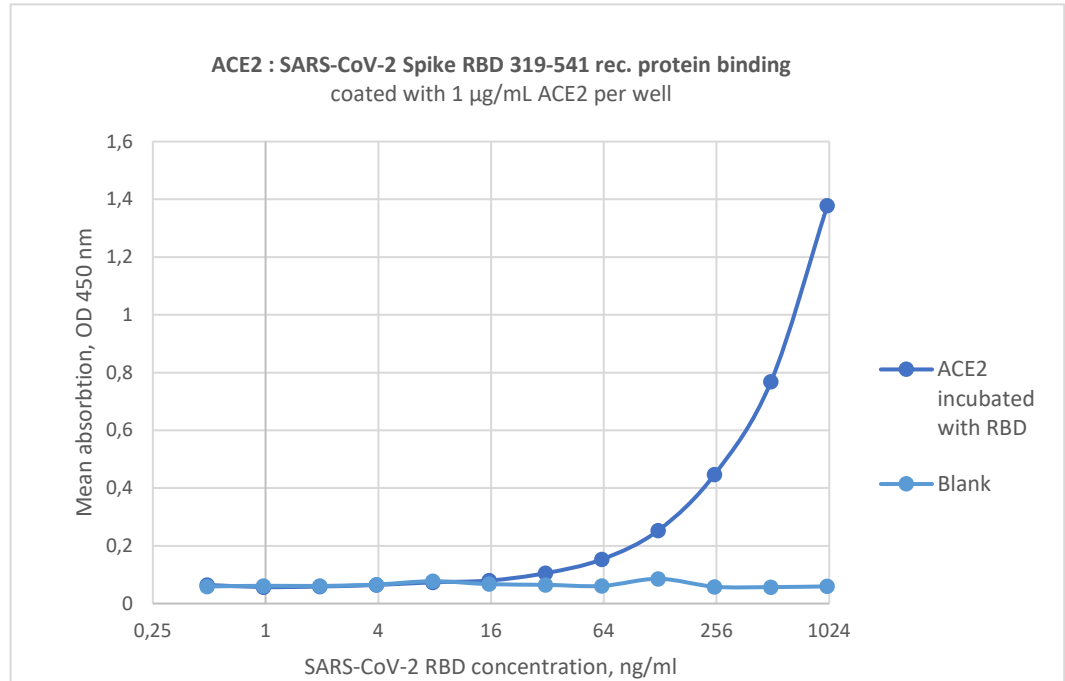
Purity: > 95% as determined by SDS-PAGE

Storage: Store at -70°C short term. Avoid freeze thaw cycles.

Bioactivity:

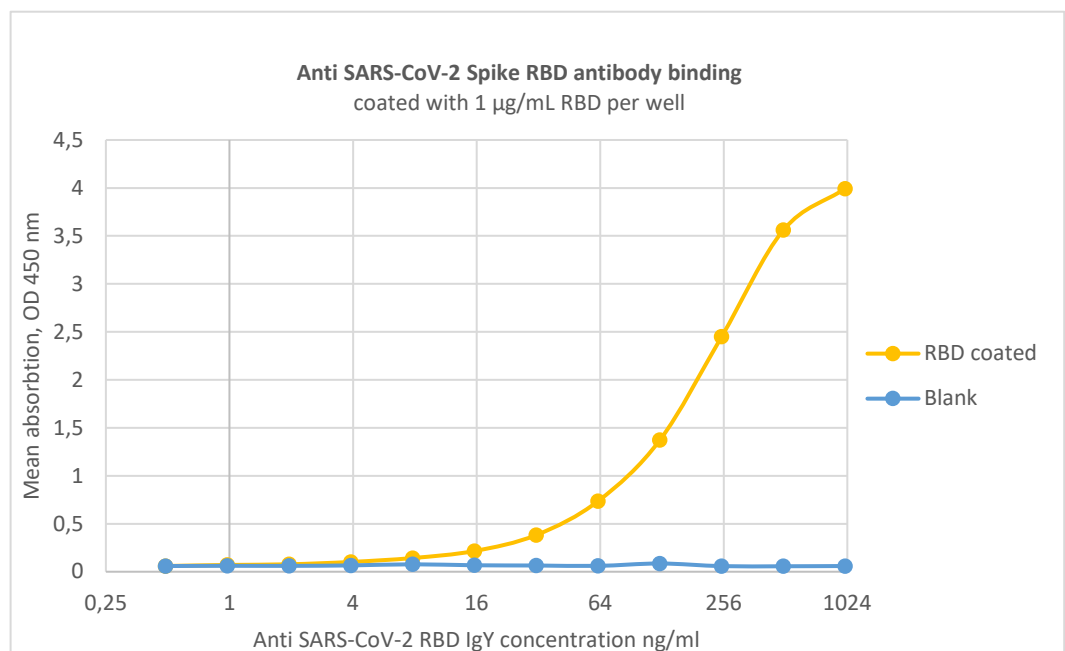
ELISA: Specificity towards recombinant ACE2 receptor

Immobilized ACE2 1 µg/mL (100µL/well) can bind SARS-CoV-2 Spike RBD 319-541 recombinant protein in a sandwich ELISA setup: ACE2 receptor + RBD recombinant antigen + anti SARS-CoV-2 Spike RBD 319-541 polyclonal IgY + Rabbit anti chicken IgG-HRP.



ELISA: High immunogenicity verified by immunization of hens.

The antigen shows strong antigenicity. Immobilized SARS-CoV-2 Spike RBD 319-541 recombinant protein at 1 µg/mL (100µL/well) binds chicken anti- SARS-CoV-2 Spike RBD 319-541 with a linear range between 64 to 256 ng/mL antibody added over fixed antigen concentration coated on the well. Starting concentration of antibody normalized to 1 µg/mL.



Mass Spectrometry analysis:

The samples have been treated with trypsin for 1h at 57°C (Tryp_CoV_2) or trypsin for 1h at 57°C followed by PNGaseF for 1h at 37°C (Tryp_PNGaseF_CoV_2). Comparison of samples provides knowledge of the glycosylation sites and sequence. Samples analyzed using Thermo Scientific™ Exploris™ 480 Mass Spectrometer.

N42(IT) and N54(AT) have been found to contain glycan structures.

Without PNGaseF

MGILPSPGMP	ALLSLVSLLS	VLLMGCVAFR	VQPTESIVRF	PNITNLCPFG	EVFNATRFAS	60
VYAWNRKRIS	NCVADYSVLY	NSASFSTFKC	YGVSPTKLND	LCFTNVYADS	FVIRGDEVQR	120
IAPGQTGKIA	DYNYKLPDDF	TGCVIAWNSN	NLDSKVGNGY	NYLYRLFRRS	NLKPFFERDIS	180
TEIYQAGSTP	CNGVEGFNCY	FPLQSYGFQP	TNGVGYQPYR	VVLSFELLH	APATVCGPKK	240
STNLVKNKCV	NFGHHHHHHH					260
Coverage: 214/260 82.31%						Spike1 Spike-sRBD 319-541

With PNGaseF

MGILPSPGMP	ALLSLVSLLS	VLLMGCVAFR	VQPTESIVRF	PNITNLCPFG	EVFNATRFAS	60
VYAWNRKRIS	NCVADYSVLY	NSASFSTFKC	YGVSPTKLND	LCFTNVYADS	FVIRGDEVQR	120
IAPGQTGKIA	DYNYKLPDDF	TGCVIAWNSN	NLDSKVGNGY	NYLYRLFRRS	NLKPFFERDIS	180
TEIYQAGSTP	CNGVEGFNCY	FPLQSYGFQP	TNGVGYQPYR	VVLSFELLH	APATVCGPKK	240
STNLVKNKCV	NFGHHHHHHH					260
Coverage: 232/260 89.23%						Spike1 Spike-sRBD 319-541