

CATALOG & PRICING

Cat. #	Size
VPS1-20	20 µg
VPS1-50	50 µg
VPS1-100	100 µg

BACKGROUND

The single-stranded, plus-sense severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome encodes nonstructural replicase polyproteins as well as structural proteins such as spike (S), nucleocapsid (N), membrane (M), and envelop (E) proteins (Zhou et al., 2020). S protein is a transmembrane homotrimeric class I fusion glycoprotein; the S1 subunit binds to angiotensin-converting enzyme II (ACE2) and S2 subunit is involved in the fusion of viral and host cell membranes (Sternberg and Naujokat, 2020). The distal subunit S1 contains a receptor binding domain (RBD, residues Arg319-Phe541, GenBank accession: YP_009724390.1), whose hinge-like conformational movement is required for ACE2 receptor binding and refolding of S2 for membrane fusion (Walls et al., 2020; Wrapp et al., 2020). Accumulating evidence from convalescent COVID-19 individuals supports that RBD is a highly immunogenic epitope targeted by neutralizing monoclonal antibodies through adaptive immune responses mediated by CD4+ T cells (Cao et al., 2020; Grifoni et al., 2020). As such, S and its RBD have been targets for vaccine design and development to prevent SARS-CoV-2 infection and rechallenge (Sternberg and Naujokat, 2020).

Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C., et al. (2020). Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells. *Cell* 182, 73-84 e16.

Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al. (2020). Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* 181, 1489-1501 e1415.

Sternberg, A., and Naujokat, C. (2020). Structural features of coronavirus SARS-CoV-2 spike protein: Targets for vaccination. *Life Sci* 257, 118056.

Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., and Velesler, D. (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 181, 281-292 e286.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367, 1260-1263.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270-273.

PRODUCT FEATURES

- Recombinant protein in human cell line HEK293
- Verified with rabbit monoclonal antibodies using contrived human nasopharyngeal swab specimens

- Can be used as an antigen to detect neutralizing antibodies in convalescent COVID-19 patient sera

PRODUCT DETAILS

Aliases: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S) receptor binding domain (RBD); 2019 novel coronavirus spike glycoprotein RBD, SARS-CoV-2 S1, SARS-CoV-2 S protein RBD; S glycoprotein RBD; S-RBD

UniProt Accession No.: P59594

NCBI Reference Sequence: YP_009724390.1

Host Species: SARS-CoV-2 spike glycoprotein (S) protein RBD is a recombinant protein produced in HEK293 cell line.

Amino Acid Sequence:

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFK
CYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWN
SNNLDSKVGGNYNLYRLFRKSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGF
QPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFHHHHHH

Purity: > 95% as established by reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Tag: Six histidine (His) residues at the C-terminus of the protein

Molecular Weight: 27 kDa

Validated Applications: Western blotting; ELISA

Storage Buffer: 0.02 M Tris buffer, pH 8.0

Storage Condition: < -20°C, stable for 1 year after receipt. Avoid repeated freeze-thaw cycles.

VALIDATION DATA

S1 (ng)	Δ RLU
250	1780301
25	121188
2.5	14453
0.25	0

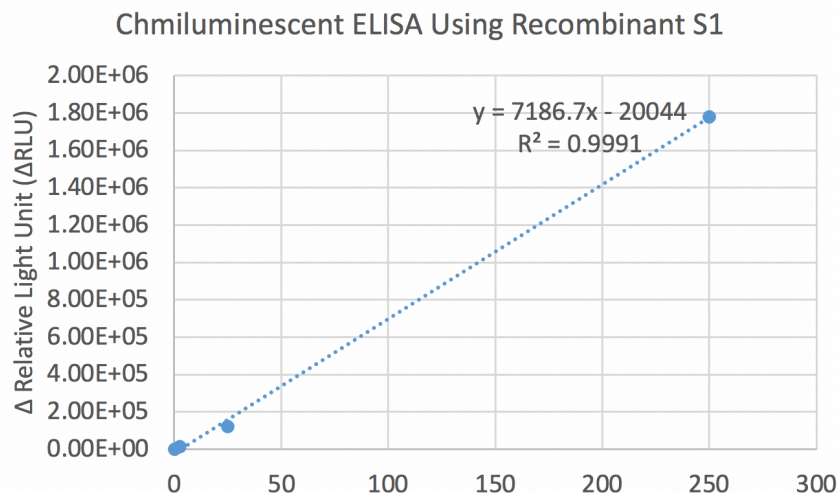


Figure 1. Chemiluminescent ELISA using recombinant SARS-CoV-2 S-RBD. Different concentrations of recombinant S-RBD (Cat# VPS1) were lysed in the Viral Lysis Buffer (Cat# VL101) and coated on microplate wells. Rabbit monoclonal anti-S-RBD antibody (Cat# VYS6, 1:10,000) and HRP-conjugated highly cross-adsorbed goat anti-rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 S-RBD protein. RLU, relative light unit. Δ RLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

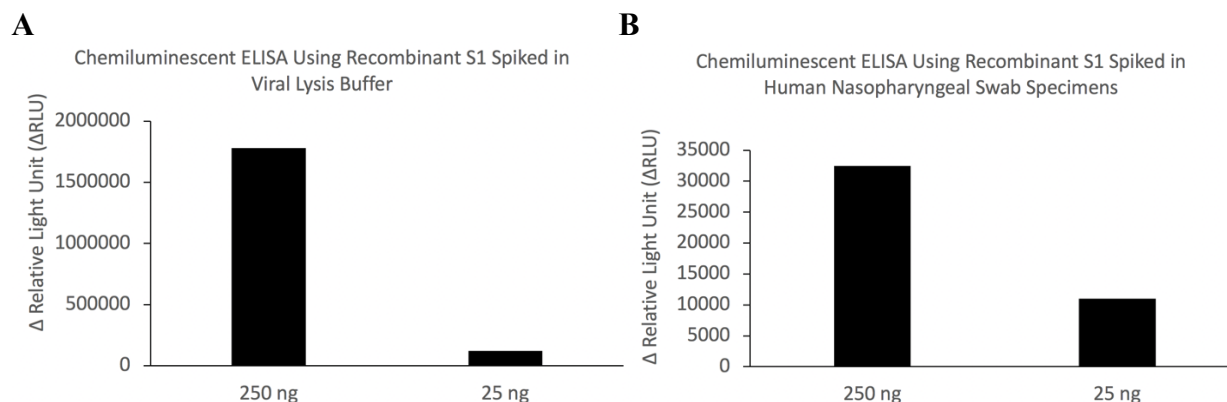


Figure 2. Clinical matrix interference test. Recombinant S-RBD (Cat# VPS1) was lysed directly in the Viral Lysis Buffer (Cat# VL101) (A) or spiked in EUA RT-PCR-confirmed negative human nasopharyngeal swab specimens before being lysed in the Viral Lysis Buffer (Cat# VL101) (B). Lysates were used to coat microplate wells. Rabbit monoclonal anti-S-RBD antibody (Cat# VYS6, 1:10,000) and HRP-conjugated highly cross-adsorbed goat anti-rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 S-RBD protein. RLU, relative light unit. Δ RLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal. Note: The clinical matrix such as nasopharyngeal swab greatly reduces the sensitivity of antigen detection.

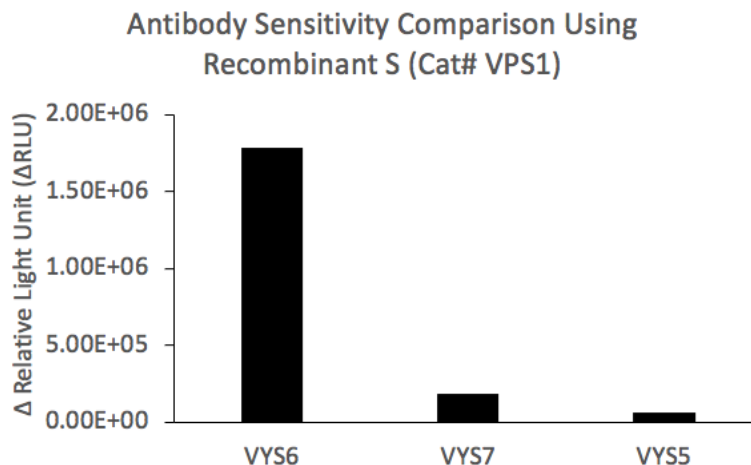


Figure 3. Comparison of sensitivities of three rabbit monoclonal antibodies. Recombinant S-RBD (Cat# VPS1, 250 ng) was lysed in the Viral Lysis Buffer (Cat# VL101) and the lysates were used to coat microplate wells. Anti-S-RBD antibodies with catalog numbers VYS6, VYS7, and VYS5 (1:10,000), and HRP-conjugated highly cross-adsorbed goat anti-rabbit IgG secondary antibody (Cat# 202, 1:20,000) were used to detect SARS-CoV-2 S-RBD protein. RLU, relative light unit. Δ RLU = RLU of the well with the primary antibody - RLU of the corresponding well without the primary antibody, everything else being equal.

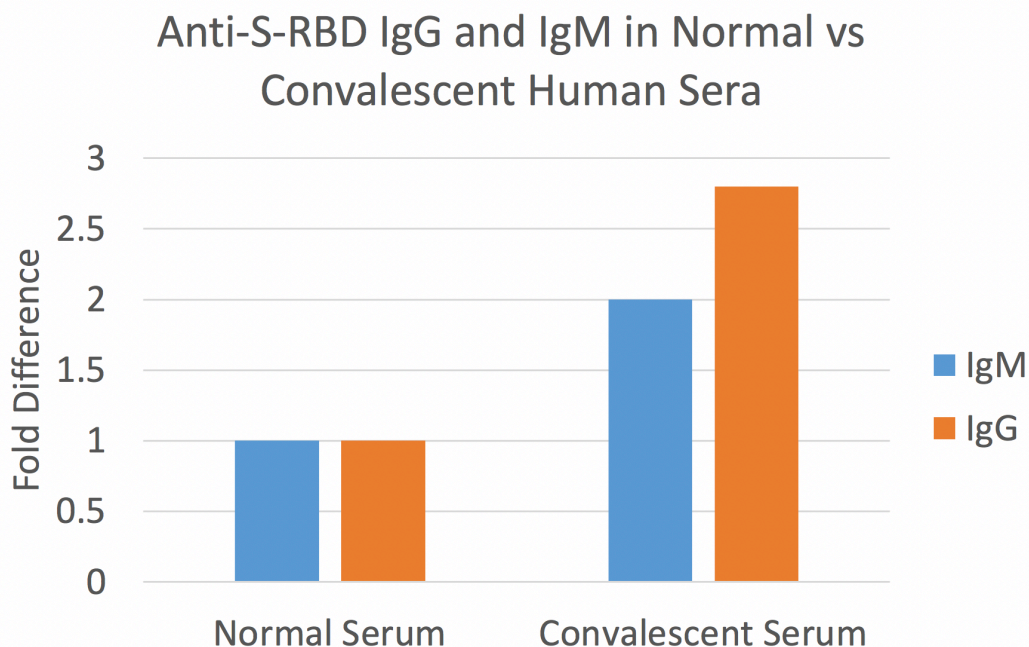


Figure 4. Serological detection of IgG and IgM against SARS-CoV-2 using recombinant S-RBD. Microplate wells were coated with 1 μ g/mL recombinant S-RBD (Cat# VPS1). Sera from a normal and a COVID-19 patient 1-month post symptom onset were diluted 1:3,000 and incubated with the coated S-RBD. PiQ™ ECL (Cat# 636) substrates were used to generate chemiluminescent signals.