Ordering Information
Web www.encorbio.com
Email admin@encorbio.com
Phone 352-372-7022
Fax 352-372-7066

HGNC Name: *NA*
UniProt: *P0DTC2*
RRID: *AB_2861175*
Immunogen: *Recombinant SARS-CoV2 S-protein ACE2 binding domain expressed in and purified from E. coli*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Stable at 4°C for one year, for longer term store at -20°C*
Recommended dilutions:
WB: 1:3,000-5,000. IF/ICC 1:3,000-5,000

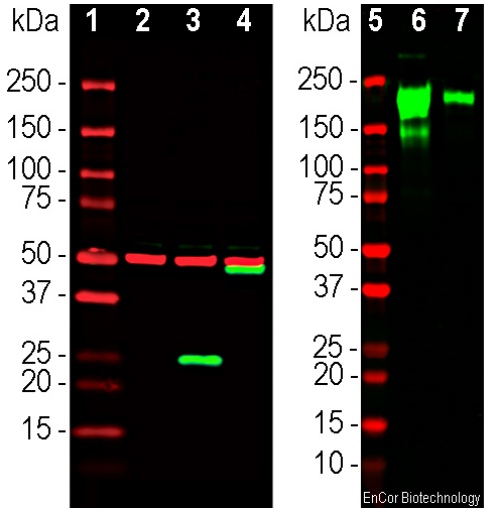
References:

1. Wu, F et al. A new coronavirus associated with human respiratory disease in China. Nature doi:10.1038/s41586-020-2008-3.2020 579:265-269 (2020).
2. Ren, L-L et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J (Engl) doi:10.1097/CM9.0000000000000722 133:1015-24 (2020).
3. Walls, A C et al. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell doi: 10.1016/j.cell.2020.02.058 180:1-12 (2020)
4. Yan, R et al. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science doi:10.1126/science.abb2762 367:1444–8 (2020).
5. Wang, D-S et al. The pleckstrin homology domain of human beta I sigma II spectrin is targeted to the plasma membrane in vivo. Biochem. Biophys. Res. Comm. 225:420-6 (1996).

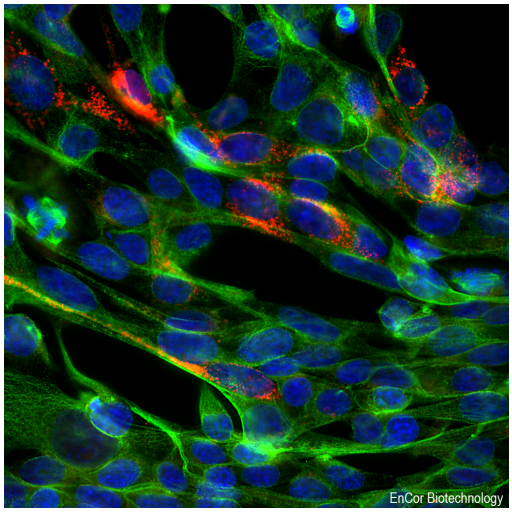
SARS-CoV2 S-protein ACE2 binding domain Rabbit Polyclonal Antibody

RPCA-SARS-CoV2-bd

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC	Rabbit	IgG1	Intact S-Protein 142kDa	NA



Left Panel: Western blot analysis of HEK293 cell lysates using rabbit pAb to SARS-CoV2-bd protein, RPCA-SARS-CoV-bd, dilution 1:2,000 in green: [1] protein standard, [2] non-transfected cells, [3] cells transfected with pCI-Neo-Mod vector containing the SARS-Cov-bd cDNA, and [4] cells transfected with pCI-Neo-GFP vector containing the SARS-CoV-bd cDNA. The band at 25kDa in lane 2 demonstrates expression of SARS-CoV-bd protein, and the band at about 50kDa in lane 3 corresponds to GFP-SARS-CoV-bd fusion protein. The same blot was simultaneously probed with EnCor mouse mAb to β-tubulin, MCA-4E4, dilution 1:5,000, in red, which reveals a single band at 50kDa in both transfected and non-transfected cells. Vector details are in reference 5. Right Panel: Blot of full length recombinant SARS-CoV2 S-protein expressed in HEK293 cells, product 10561-CV, from R&D Systems. Lane 6 shows a loading of 1µg and lane 7 is 100ng. On longer exposure of the blot the antibody could readily detect 10ng of the S-protein. Lanes 1 and 5 are molecular weight standards of indicated size.



Immunofluorescent analysis of HEK293 cells transfected with pCI-Neo-Mod vector (5) including DNA encoding the SARS-CoV2 S-protein ACE2 binding domain, amino acids 308-541 in the S-protein sequence in Wuhan-Hu-1, complete genome. This region was used as the immunogen for development of rabbit antibody RPCA-SARS-CoV2-bd. The antibody was used at a dilution 1:3,000, shown in red. Cells costained with EnCor mouse mAb to β-tubulin, MCA-4E4, dilution 1:5,000, in green. The blue is Hoechst staining of nuclear DNA. The RPCA-SARS-CoV2-bd antibody cleanly reveals expression of the SARS-CoV2-bd protein only in transfected cells. The β-tubulin antibody produces strong staining of microtubules in the cytoplasm in both transfected and non-transfected cells.

Background:

In late 2019 a novel infectious disease was discovered in Wuhan, China which was quickly recognized to be caused by a previously unknown RNA coronavirus. The virus was very rapidly isolated, the full RNA sequence determined and put on-line on the 10th of January 2020. The sequence revealed that the virus was most closely related to certain bat coronaviruses and the severe acute respiratory syndrome (SARS) coronavirus. Immediately biotechnology companies and research institutes used the RNA sequence information to generate vaccine candidates. The SARS virus was known to enter and infect human cells by means of the so-called spike or S-protein which binds to the extracellular domain of the angiotensin converting enzyme 2 (ACE2) protein, which is then internalized bringing the virus into the cell. Cryoelectron microscopy and binding studies quickly determined that the S-protein of SARS-CoV2 is structurally similar to to that of the SARS virus and also binds to the ACE2 receptor, albeit with higher affinity than the S-protein of SARS. This focuses attention on the ACE2 binding site on the SARS-CoV2 S-protein and for the complementary region on ACE2 which binds the SARS-CoV2 S-protein. We therefore expressed both these regions in *E. coli*, our products PROT-SARS-CoV2-bd and PROT-ACE2-bd and raised antibodies to them.

The RPCA-SARS-CoV2-bd antibody was made against our recombinant construct comprising amino acids 308-541 in the S-protein sequence in SARS-CoV2 Wuhan-Hu-1, complete genome. The antibody works well on western blots of crude homogenates of HEK293 cells transfected with the SARS-CoV2 binding domain, cleanly producing the appropriate sized band and as expected also binds the full length S-protein. In addition S-protein transfected cells and cells infected with patient derived SARS-CoV2 show clean and strong immunofluorescence staining of transfected or infected cells. EnCor also supplies two mouse monoclonal antibodies to the SARS-CoV2 ACE2 binding domain MCA-5G8 and MCA-2G1.

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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