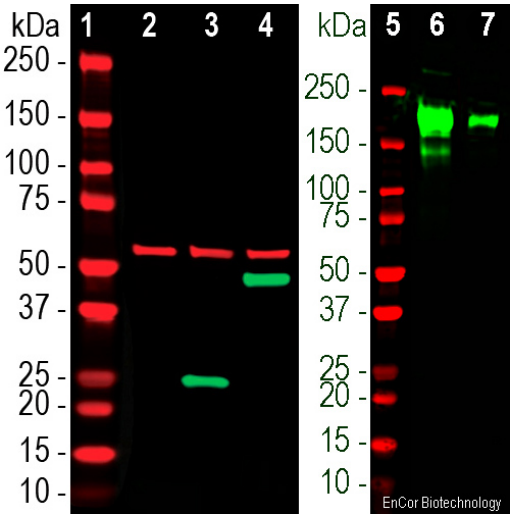
SARS-CoV2 S-protein ACE2 binding domain Mouse Monoclonal Antibody

MCA-2G1

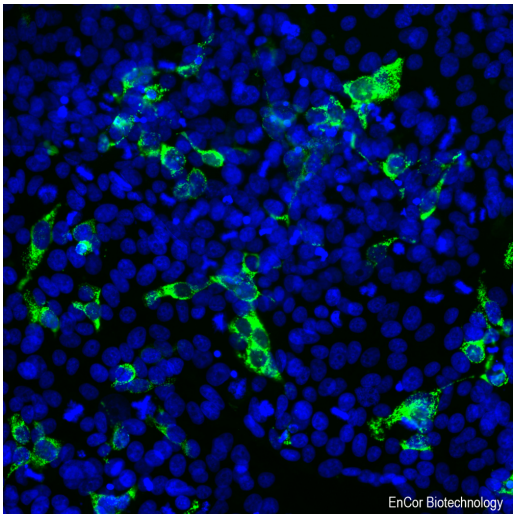
Ordering Information
Web *www.encorbio.com*
Email *admin@encorbio.com*
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *NA*
UniProt: *P0DTC2*
RRID: *AB_2861173*
Immunogen: *Recombinant SARS-CoV2 S-Protein ACE2 binding domain expressed in and purified from E. coli, EnCor product PROT-r-SARS-CoV2-bd*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Shipped on ice. Store at 4°C for short term, for longer term at -20°C. Avoid freeze / thaw cycles.*
Recommended dilutions:
WB: 1:1,000-1:3,000. ICC/IF: 1:1,000

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



Left Panel: HEK293 cells were transfected with DNA encoding the S-protein ACE2 binding site in PROT-SARS-CoV2-bd which was inserted into pCI-Neo-Mod or pCI-Neo-GFP eukaryotic expression vectors, which express either the insert only or the insert fused with GFP (5). Lane 2 shows a crude homogenate of untransfected control cells, lane 3 shows a homogenate of cells expressing SARS-CoV2-bd and lane 4 shows a homogenate of cells expressing GFP-SARS-CoV2-bd fusion. The MCA-2G1 was used at a dilution of 1:3,000 and produces clean and specific staining of bands of the expected size as shown in green. The blot was also stained with EnCor rabbit polyclonal control antibody to HSP60, RPCA-HSP60, at a dilution of 1:5,000 in red. Right Panel: Blot of full length recombinant SARS-CoV2 S-protein expressed in HEK293 cells, product 10561-CV, obtained 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.



HEK293 cells were transfected with the DNA encoding the S-protein segment in PROT-SARS-CoV2-bd which was inserted in the pCI-Neo-Mod expression vector (5). The MCA-2G1 produces clean and specific staining of transfected cells which stain identically with RPCA-SARS-CoV2-bd, our rabbit polyclonal antibody to the same immunogen. The nuclei of transfected and untransfected cells are shown in blue with DAPI DNA stain.

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-R-SARS-CoV2-bd and PROT-R-ACE2-bd and raised antibodies to them.

The MCA-2G1 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 show clean and strong immunofluorescence staining of the expressed protein with this antibody. We are currently determining the exact peptide epitope of this and our other SARS-CoV2 S-protein antibodies and also measuring their kinetic properties. EnCor supplies another mouse monoclonal antibody to the SARS-CoV2 S-protein ACE2 binding domain MCA-5G8 and also a rabbit polyclonal RPCA-SARS-CoV2-bd.

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 β-I σ-II spectrin is targeted to the plasma membrane in vivo. Biochem. Biophys. Res. Comm. 225:420-6 (1996).

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.

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.