PROTEINS AND ANTIBODIES FOR SARS-COV-2 RESEARCH

bio-techne®

PROTEINS FOR SARS-COV-2 RESEARCH

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the ongoing COVID-19 global pandemic has resulted in the urgent need to understand the pathogenicity of the virus. As the SARS-CoV-2 Spike protein is a key factor regulating viral attachment and fusion of the viral and host cell membranes, it is a primary target for therapeutic research and vaccine development. To help facilitate this research, Bio-Techne now offers a wide selection of R&D Systems® bioactive, recombinant SARS-CoV-2 Spike proteins, including the **SARS-CoV-2 Spike protein receptor binding domain (RBD)**, the full ectodomain **SARS-CoV-2 Spike active trimer**, and both the **Spike S1** and **S2** subunit proteins, along with recombinant human **ACE-2**. We also now offer the **SARS-CoV-2 papain-like protease**, and the **3CL protease**, which are considered to be additional promising therapeutic targets for COVID-19.

Visit us online to view a complete listing of our Recombinant Proteins for Coronavirus Research | rndsystems.com/products/proteins-coronavirus-research

ADVANTAGES OF USING R&D SYSTEMS PROTEINS FOR SARS-COV-2 RESEARCH

OUR CORONAVIRUS-RELATED PROTEINS ARE MANUFACTURED BY R&D SYSTEMS SCIENTISTS

All R&D Systems coronavirus-related proteins are produced and purified by our experienced in-house scientists, allowing us to maintain complete control over the quality of our products.

BIOACTIVITY TESTING

The bioactivity of each protein is tested in an appropriate biological system, including high affinity binding assays to ACE-2 for the SARS-CoV-2 Spike proteins and enzymatic reporter assays for the coronavirus proteases.

LOT-TO-LOT CONSISTENCY TESTING

Maintaining consistent manufacturing conditions and testing each new lot side-by-side with previous lots guarantees that the new lot displays the same level of bioactivity, purity, and endotoxin levels as previous lots.

ADDITIONAL ANALYTICAL TESTING DATA AVAILABLE

Size exclusion chromatography (SEC), mass spectrometry, capillary isoelectric focusing (cIEF), differential scanning fluorimetry (DSF), dynamic light scattering (DLS), and surface plasmon resonance (SPR) are used to provide supporting evidence that a specific protein is the correct size or structure and displays the correct binding properties.

MULTIPLE HOST EXPRESSION SYSTEMS, TAGS, AND LABELS

Host expression systems include insect cells, CHO cells, and HEK293 cells. Proteins with His tags, Fc tags, as well as amine biotinylated and Avi-tag biotinylated proteins are available.

SCALABILITY

R&D Systems has developed high yielding production procedures that enable scalable production capabilities to meet the demands required for assay development, manufacturing, and therapeutic quality control testing.

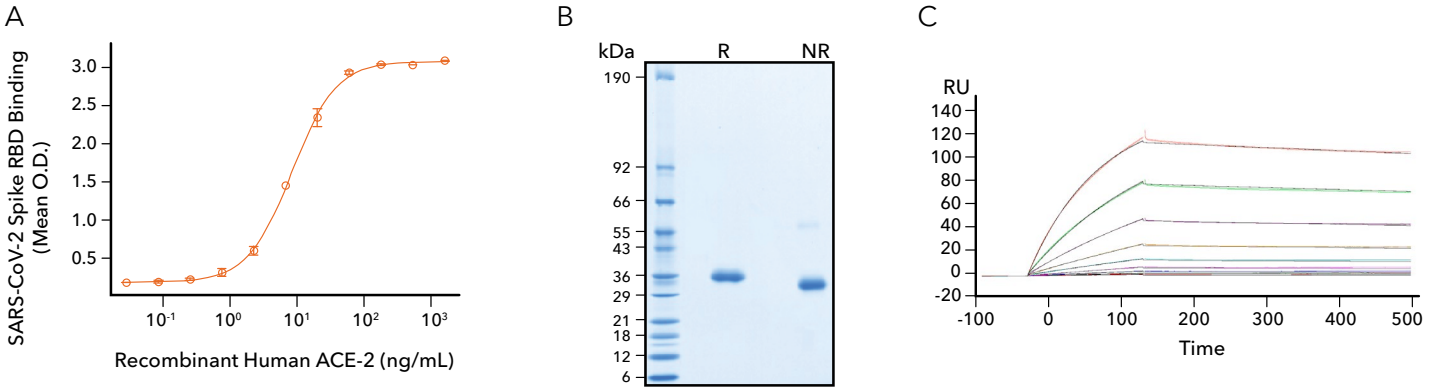
CUSTOM PROTEIN CAPABILITIES

Whether it's developing a protein from scratch or customizing one from our catalog, our experienced team will work with you to create the protein that fits your experimental needs.

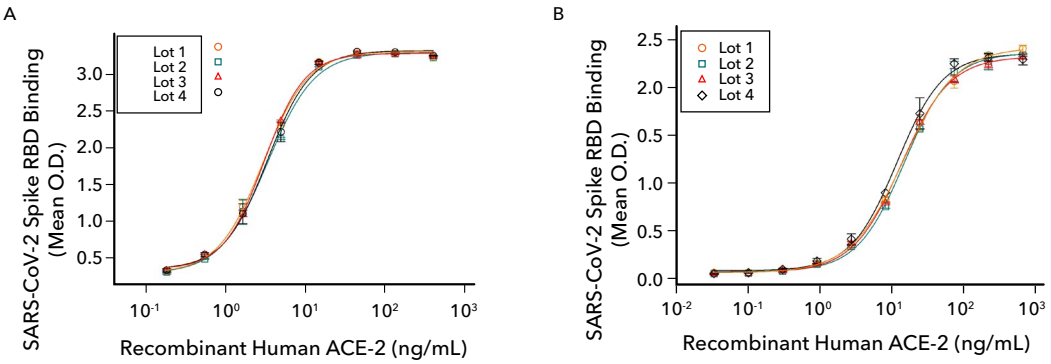
If you have any concerns about the reliability of your current supply of proteins for SARS-CoV-2 research, you can count on us to provide you with the key proteins that you need to keep your research going. We have the expertise and the capability to scale up the production of any protein and our products are developed with great care and backed by extensive bioactivity and analytical testing data.

BIOACTIVITY TESTING OF R&D SYSTEMS SARS-COV-2 SPIKE PROTEINS

The data examples shown below demonstrate the bioactivity and analytical testing that we have performed on our SARS-CoV-2 Spike proteins, along with examples of our lot-to-lot consistency testing using either the recombinant SARS-CoV-2 Spike RBD protein or recombinant human ACE-2. The data shown is typical of the analyses that R&D Systems scientists routinely perform to characterize our recombinant proteins prior to their commercial release.



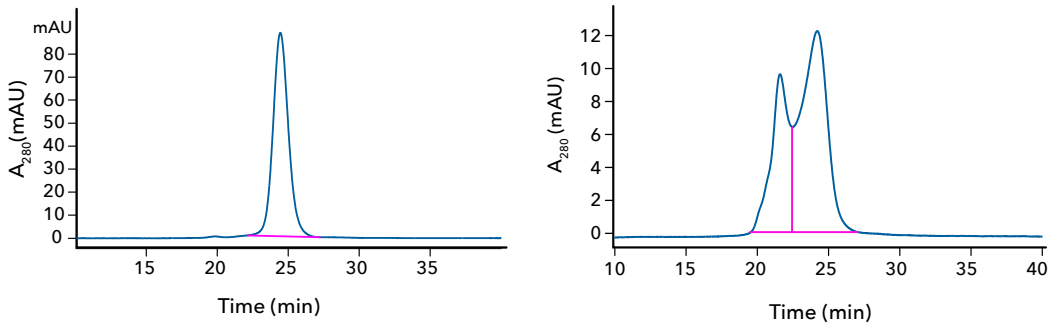
Bioactivity and Purity Testing of Recombinant SARS-CoV-2 Spike RBD His-tag Protein. (A) Recombinant SARS-CoV-2 Spike RBD His-tag Protein (HEK293-expressed; Catalog # 10500-CV) binds to Recombinant Human ACE-2 His-tag (Catalog # 933-ZN) in a functional ELISA. **(B)** The purity of Recombinant SARS-CoV-2 Spike RBD His-tag Protein (HEK293-expressed; Catalog # 10500-CV) was assessed by SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 32-38 kDa.**(C) Binding of ACE-2 to SARS-CoV-2 Spike RBD by Surface Plasmon Resonance (SPR).** Recombinant SARS-CoV-2 Spike RBD His-tag Protein (Catalog 3 10500-CV) was immobilized on a Biacore Sensor Chip CM5, and binding to Recombinant Human ACE-2 (Catalog # 933-ZN) was measured at a concentration range between 0.37 nM and 93.5 nM. The double-referenced sensorgram was fit to a 1:1 binding model to determine the binding kinetics and affinity, with an affinity constant of KD = 2.149 nM (Biacore T200).



Bioactivity and Lot-to-Lot Consistency Testing of Recombinant SARS-CoV-2 Spike RBD Protein and Recombinant Human ACE-2. (A) Four independent lots of Recombinant SARS-CoV-2 Spike RBD His-tag Protein (HEK293-expressed; Catalog # 10500-CV) were tested for their ability to bind to Recombinant Human ACE-2 His-tag Protein (Catalog # 933-ZN) in a functional ELISA. The data demonstrates that the Recombinant SARS-CoV-2 Spike RBD Protein binds to Recombinant Human ACE-2 and that the four different lots of the SARS-CoV-2 Spike RBD protein display lot-to-lot consistency. **(B)** Four independent lots of Recombinant Human ACE-2 His-tag Protein (Catalog # 933-ZN) were tested for their ability to bind to Recombinant SARS-CoV-2 Spike RBD Fc Chimera Protein (Catalog # 10499-CV) in a functional ELISA. The data demonstrates that the Recombinant Human ACE-2 Protein binds to the Recombinant SARS-CoV-2 Spike RBD Protein and that the four different lots of the ACE-2 protein display lot-to-lot consistency.

EXAMPLES OF ADDITIONAL ANALYTICAL TESTING PERFORMED ON R&D SYSTEMS SARS-COV-2 SPIKE PROTEINS

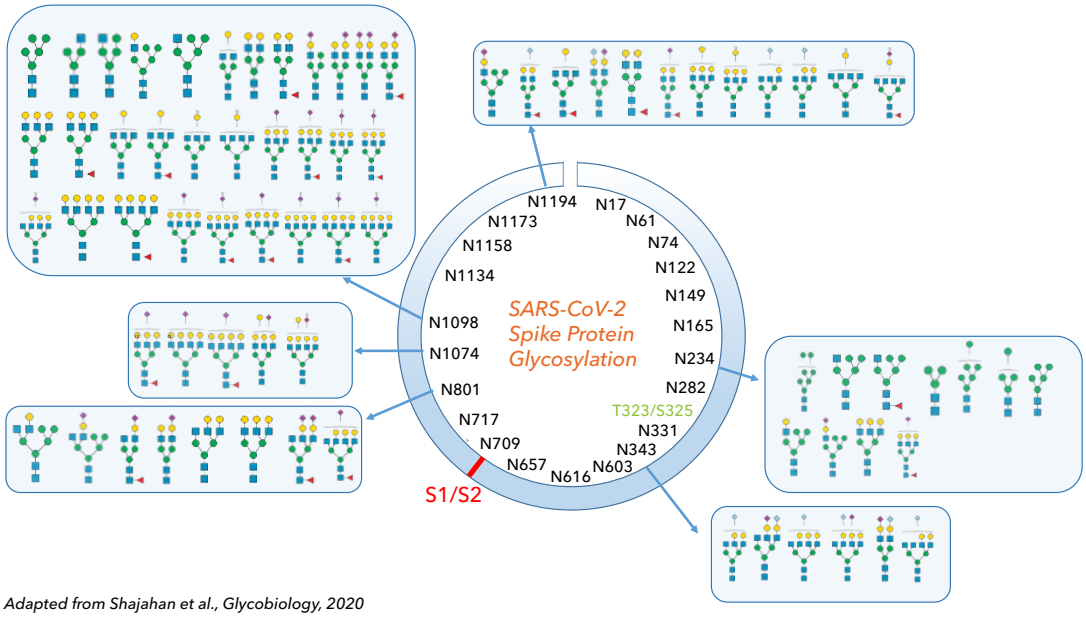
We have also performed additional analytical testing on our SARS-CoV-2 Spike proteins when appropriate. The data examples below show analysis of our recombinant SARS-CoV-2 Spike Active Trimer and Spike (GCN4-IZ) proteins by size exclusion chromatography.



Analysis of SARS-CoV-2 Spike Proteins by Size Exclusion Chromatography. (A) Recombinant SARS-CoV-2 Spike Protein Active Trimer His-tag Protein (HEK293-expressed; Catalog # 10549-CV) was analyzed by size exclusion chromatography (SEC) resulting in a single major peak. The predicted MW was calculated to be 373kDa, consistent with a trimeric conformation. **(B)** Recombinant SARS-CoV-2 Spike (GCN4-IZ) His-tag Protein (HEK293-expressed; Catalog # 10561-CV) was analyzed by size exclusion chromatography resulting in two major peaks. The predicted MW of the larger peak was calculated to be 391kDa, consistent with a trimeric conformation. A second peak of a higher MW was also detected. This peak corresponds to a higher order oligomer, likely a hexamer, due the GCN4-IZ trimerization domain.

GLYCOSYLATION FOOTPRINTING

The SARS-CoV-2 Spike protein has been reported to be extensively glycosylated with host-derived glycans at 22 N-linked glycosylation sites and at least 2 O-glycan sites. These modifications increase both the stability and solubility of the Spike protein and camouflage its immunogenic epitopes, enhancing the virus' ability to evade the host immune response. Based on these observations, we further characterized our SARS-CoV-2 Spike RBD proteins produced from insect cells, HEK293 cells, and CHO cells by analyzing their glycosylation using direct fluorescent glycan labeling tools.



Adapted from Shajahan et al., Glycobiology, 2020

Analysis of the Glycosylation of the SARS-CoV-2 Spike Protein. Recombinant SARS-CoV-2-Spike Protein Active Trimer (Catalog 10549-CV) was reduced, alkylated, and digested with trypsin prior to analysis using a Thermo Scientific™ Vanqish UHPLC coupled with a Thermo Scientific™ Q Exactive HF Quadrupole-Orbitrap™ Mass Spectrometer operating in data-dependent scanning mode. Data was analyzed using Thermo Scientific™ BioPharma Finder software, using the CHO glycosylation database.

To learn more about SARS-CoV-2 Spike protein glycosylation, please see our recent application note, *Glycosylation of the receptor binding domain of COVID-19 virus spike protein* | rndsystems.com/resources/articles/glycosylation-receptor-binding-domain-covid-19-virus-spike-protein

RECOMBINANT PROTEINS FOR CORONAVIRUS RESEARCH

SARS-CoV-2 SPIKE PROTEINS				
PROTEIN	SOURCE	TAG	CATALOG #	DESCRIPTION
SARS-CoV-2 Spike Protein RBD	HEK293	Fc	10499-CV	The receptor binding domain (RBD) of the SARS-CoV-2 Spike protein that binds with high affinity to the host cell receptor, ACE-2.
	HEK293	His	10500-CV	
	CHO	Fc	10542-CV	
	CHO	His	10534-CV	
	*Sf*21 (baculovirus)	Fc	10565-CV	
	Tn5 (baculovirus)	His	10523-CV	
SARS-CoV-2 Spike Protein RBD, Biotinylated	HEK293	Avi, Biotin, Fc	AVI10499; Please inquire	A biotinylated version of the receptor binding domain (RBD) of the SARS-CoV-2 Spike protein that binds with high affinity to the host cell receptor, ACE-2.
	HEK293	His	BT10500	
SARS-CoV-2 Spike Protein (Active Trimer)	HEK293	His	10549-CV	The SARS-CoV-2 ectodomain, trimeric conformation; Stabilizing mutations, K986P and V987P, promote prefusion conformation; Two additional mutations, R682S and R685S, eliminate a Furin protease cleavage site.
	CHO	His	10586-CV	
SARS-CoV-2 Spike Protein (Active Trimer), Biotinylated	HEK293	His	BT10549; Please inquire	A biotinylated version of the SARS-CoV-2 ectodomain, trimeric conformation; Stabilizing mutations, K986P and V987P, promote prefusion conformation; Two additional mutations, R682S and R685S, eliminate a Furin protease cleavage site.
SARS-CoV-2 Spike Protein (GCN4-IZ)	HEK293	His	10561-CV	The SARS-CoV-2 ectodomain with a GCN4 isoleucine zipper trimerization domain; Stabilizing mutations, K986P and V987P, promote prefusion conformation; Two additional mutations, R682S and R685S, eliminate a Furin protease cleavage site.
SARS-CoV-2 Spike Ectodomain (D614G; Active Trimer)	HEK293	His	10587-CV	The SARS-CoV-2 ectodomain, trimeric conformation with a D614G amino acid change that has been found to be associated with greater infectivity and higher viral load and is the most prevalent form in the global pandemic; Also contains the stabilizing mutations, K986P and V987P, which promote prefusion conformation, and two additional mutations, R682S and R685S, which eliminate a Furin protease cleavage site.
SARS-CoV-2 Spike S1 Subunit Protein	HEK293	His	10569-CV	The S1 subunit of the SARS-CoV-2 Spike protein that is generated upon proteolytic cleavage of the Spike protein into the S1 and S2 subunits, which is required for activation. The S1 subunit is involved in attachment of the protein to the host cell receptor.
	*Sf*21 (baculovirus)	His	10522-CV	
SARS-CoV-2 Spike S1 Subunit Protein, Biotinylated	HEK293	His	BT10569; Please inquire	A biotinylated version of the S1 subunit of the SARS-CoV-2 Spike protein that is generated upon proteolytic cleavage of the Spike protein into the S1 and S2 subunits, which is required for activation. The S1 subunit is involved in attachment of the protein to the host cell receptor.
SARS-CoV-2 Spike S2 Subunit Protein	Tn5 (baculovirus)	His	10584-CV	The S2 subunit of the SARS-CoV-2 Spike protein that is generated upon proteolytic cleavage of the Spike protein into the S1 and S2 subunits, which is required for activation. The S2 subunit is involved in cell fusion.
SARS-CoV-2 Spike S2 Subunit Protein (GCN4-IZ)	HEK293	His	10590-CV; Please inquire	The S2 subunit of the SARS-CoV-2 Spike protein that is generated upon proteolytic cleavage of the Spike protein into the S1 and S2 subunits, with a GCN4 isoleucine zipper trimerization domain. Proteolytic cleavage of the Spike protein is required for activation, with the S2 subunit being involved in cell fusion.

SARS-CoV SPIKE PROTEINS				
PROTEIN	SOURCE	TAG	CATALOG #	DESCRIPTION
SARS-CoV Spike RBD Protein	HEK293	Fc	10582-CV	The receptor binding domain (RBD) of the SARS-CoV-1 Spike protein that binds with high affinity to the host cell receptor, ACE-2.
	HEK293	His	10558-CV	
	CHO	Fc	10559-CV	
	CHO	His	10558-CV	
SARS-CoV Spike (GCN4-IZ)	CHO	His	10581-CV	The SARS-CoV-1 ectodomain with a GCN4 isoleucine zipper trimerization domain; Stabilizing mutations, K968P and V969P, promote prefusion conformation.
SARS-CoV Spike S1 Subunit Protein	*Sf*21 (baculovirus)	His	10570-CV	The S1 subunit of the SARS-CoV-1 Spike protein that is generated upon proteolytic cleavage of the Spike protein into the S1 and S2 subunits, which is required for activation. The S1 subunit is involved in attachment of the protein to the host cell receptor.

MERS-CoV SPIKE PROTEINS				
PROTEIN	SOURCE	TAG	CATALOG #	DESCRIPTION
MERS-CoV Spike S1 Subunit Protein	CHO	Fc	10606-CV	The S1 subunit of the MERS-CoV Spike protein that is generated upon proteolytic cleavage of the Spike protein into the S1 and S2 subunits, which is required for activation. The S1 subunit is involved in attachment of the protein to the host cell receptor.

NUCLEOCAPSID PROTEINS				
PROTEIN	SOURCE	TAG	CATALOG #	DESCRIPTION
SARS-CoV-2 Nucleocapsid Protein	*Sf*21	His	10474-CV	The SARS-CoV-2 Nucleocapsid protein, which is abundant during infection and displays high immunogenic activity, making it a promising therapeutic target.
MERS-CoV Nucleocapsid Protein	*Sf*21	His	10521-CV	The MERS-CoV Nucleocapsid protein, which is abundant during infection and displays high immunogenic activity, making it a promising therapeutic target.

CORONAVIRUS PROTEASES				
PROTEIN	SOURCE	TAG	CATALOG #	DESCRIPTION
SARS-CoV-2 Papain-like Protease	*E. coli*	GST	E-611	Recombinant SARS-CoV-2 virus Papain-like protease (PLPro) is a Ubiquitin- and ISG15-deconjugating enzyme.
SARS-CoV-2 3CL Protease	*E. coli*	None	E-720	Recombinant SARS-CoV-2 3CL Protease is a cysteine protease that cleaves proteins with sequences including LQ[S/A/G). The cleavage occurs C-terminal to the glutamine residue.
SARS-CoV Papain-like Protease	*E. coli*	His	E-610	Recombinant SARS-CoV-1 virus Papain-like protease (PLPro) is a Ubiquitin- and ISG15-deconjugating enzyme.
SARS-CoV 3CL Protease	*E. coli*	None	E-718	Recombinant SARS-CoV-1 3CL Protease is a cysteine protease that cleaves proteins with sequences including LQ[S/A/G). The cleavage occurs C-terminal to the glutamine residue.
MERS-CoV Papain-like Protease	*E. coli*	His	E-609	Recombinant MERS-CoV virus Papain-like protease (PLPro) is a Ubiquitin- and ISG15-deconjugating enzyme.
MERS-CoV 3CL Protease	*E. coli*	None	E-719	Recombinant MERS-CoV 3CL Protease is a cysteine protease that cleaves proteins with sequences including LQ[S/A/G). The cleavage occurs C-terminal to the glutamine residue.

For additional information on the SARS-CoV-2 Papain-like Protease and 3CL Protease, please see our recent article, *SARS-CoV-2 3CL and PLPro: Key Protease Targets for COVID-19* | rndsystems.com/resources/articles/3cl-plpro-key-protease-targets-covid19

HUMAN ACE-2 PROTEINS			
PROTEIN	SOURCE	TAG	CATALOG #
ACE-2	NS0	His	933-ZN
	CHO	Fc	10544-ZN
	CHO	Avi, Biotin, Fc	AVI10544
	CHO	Avi, Biotin, His	AVI10579
	NS0	Biotin, His	BT933

For the most up-to-date listing of available proteins, please visit the Proteins for Coronavirus Research page on our website | rndsystems.com/products/proteins-coronavirus-research

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ANTIBODIES FOR SARS-COV-2 RESEARCH

Bio-Techne offers both R&D Systems® and Novus Biologicals® antibodies that are qualified for multiple different applications as indicated on our datasheets. We offer a large collection of antibodies for SARS-CoV-2 research, including antibodies for detecting **SARS-CoV-2 Spike, Nucleocapsid, Membrane**, or **Envelope** proteins, **SARS-CoV-2 3CL protease**, and **ACE-2**, along with **SARS-CoV-2 Spike RBD** and **ACE-2** blocking antibodies.

ADVANTAGES OF BIO-TECHNE ANTIBODIES FOR SARS-COV-2 RESEARCH

SPECIFIC AND REPRODUCIBLE

Bio-Techne antibodies are tested in the applications listed on our websites and datasheets. We ensure lot-to-lot consistency with our Quality Control requirements.

100% GUARANTEED

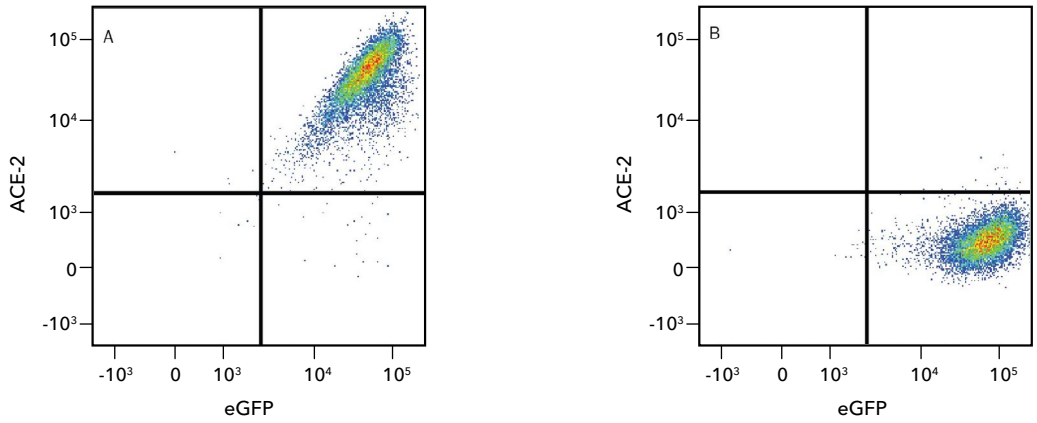
Bio-Techne antibodies are guaranteed to perform according to the specifications and applications on our datasheets.

APPLICATION-BASED DATA

Bio-Techne antibodies have application-based data demonstrating their high-level of performance. This information can easily be found on our website product pages and product datasheets.

ANTIBODIES FOR DETECTING SARS-COV-2 AND ACE-2

To detect the expression of SARS-CoV-2 in cells or tissues and further our understanding of the cell types targeted by the virus, we offer antibodies against the SARS-CoV-2 Spike RBD, the Spike S1 or S2 subunits, the SARS-CoV-2 Nucleocapsid, Membrane, or Envelope proteins, and ACE-2, which are validated for one or more of the following applications: IHC, Western blot, Simple Western, CyTOF, or flow cytometry.



Detection of ACE-2 in the HEK293 Human Cell Line Transfected with Human ACE-2 and eGFP by Flow Cytometry. The HEK293 human embryonic kidney cell line transfected with (A) human ACE-2 or (B) an irrelevant protein, and eGFP. The cells were then stained with an Alexa Fluor 647-conjugated Mouse Anti-Human ACE-2 Monoclonal Antibody (R&D Systems, Catalog # FAB9332R). Quadrant markers were set based on staining with an Alexa Fluor 647-conjugated Mouse IgG2A Isotype Control (R&D Systems, Catalog # IC003R; data not shown). Staining was performed using our Staining Membrane-Associated Proteins protocol.

ANTIBODIES FOR SARS-COV-2 RESEARCH

ANTIBODIES FOR SARS-COV-2 OR ACE-2 DETECTION				
PRODUCT	BRAND	CATALOG #	APPLICATIONS	FLUOROCHROME-CONJUGATED ANTIBODIES (CATALOG # - FLUOROCHROME)
Human/Rat/Hamster ACE-2 Antibody	R&D Systems	MAB9332	FC, IHC, CyTOF-ready	FAB9332-G, N, P, R, S, T, U, V

Human ACE-2 Antibody	R&D Systems	MAB9333	FC, CyTOF-ready	FAB9333-G, N, R, S, T, U, V
Human ACE-2 Antibody	R&D Systems	MAB9334	FC, CyTOF-ready	FAB9334-G, N, R, S, T, U, V
Human/Mouse/Rat/Hamster ACE-2 Antibody	R&D Systems	AF933	B/N, FC, IHC, IP, SW	FAB933-A, G, N, P, R, S, T, U, V
SARS-CoV-2 Spike S1 Subunit Antibody	R&D Systems	MAB105403	FC, IHC, CyTOF-ready, WB	FAB105403-G, N, R, S, T, U, V
SARS-CoV-2 Nucleocapsid Antibody	R&D Systems	MAB10474	IHC, WB	
SARS-CoV-2 Nucleocapsid Antibody	R&D Systems	MAB104741	IHC, WB	

Application Key: **B/N** Blocking/Neutralization, **CyTOF-ready** Mass Cytometry, **E** ELISA, **FC** Flow Cytometry, **IHC** Immunohistochemistry, **IP** Immunoprecipitation, **SW** Simple Western **WB** Western Blot

Fluorochrome Key: **A**: Allophycocyanin; **G**: Alexa Fluor® 488; **N**: Alexa Fluor® 700; **P**: Phycoerythrin; **R**: Alexa Fluor® 647; **S**: Alexa Fluor® 750; **T**: Alexa Fluor® 594; **U**: Alexa Fluor® 350; **V**: Alexa Fluor® 405

For the most up-to-date product listing of available antibodies, please visit the Flow Cytometry Workflows for COVID-19 Research page on our website | rndsystems.com/products/flow-cytometry-workflows-for-covid-19-research

PRODUCT	BRAND	CATALOG #	APPLICATIONS
SARS-CoV-2 Envelope Antibody	Novus Biologicals	NBP2-41061	E, IHC, IF
SARS-CoV-2 Envelope Antibody - C-terminus	Novus Biologicals	NBP3-05699	WB, E
SARS-CoV-2 Membrane Protein Antibody	Novus Biologicals	NBP3-05698	WB, E
SARS-CoV-2 Membrane Protein Antibody - C-terminus	Novus Biologicals	NBP3-05711	WB, E
SARS-CoV-2 ORF10 Antibody	Novus Biologicals	NBP3-05710	WB, E
SARS-CoV-2 ORF3a Antibody	Novus Biologicals	NBP3-05731	WB, E
SARS-CoV-2 ORF3a Antibody - N-Terminus	Novus Biologicals	NBP3-05708	WB, E
SARS-CoV-2 ORF3a Antibody - N-Terminus	Novus Biologicals	NBP3-05709	WB, E
SARS-CoV-2 ORF3a Antibody - N-Terminus	Novus Biologicals	NBP3-05719	WB, E
SARS-CoV-2 ORF6 Antibody	Novus Biologicals	NBP3-05707	WB, E
SARS-CoV-2 ORF7a Antibody - C-terminus	Novus Biologicals	NBP3-05733	WB, E
SARS-CoV-2 ORF7a Antibody - N-Terminus	Novus Biologicals	NBP3-05718	WB, E
SARS-CoV-2 ORF8 Antibody	Novus Biologicals	NBP3-05720	WB, E
SARS-CoV-2 ORF8 Antibody	Novus Biologicals	NBP3-05732	WB, E
SARS-CoV-2 ORF8 Antibody - C-terminus	Novus Biologicals	NBP3-05734	WB, E
SARS-CoV-2 3CL Protease Antibody	Novus Biologicals	NBP3-05715	WB, E
SARS-CoV-2 3CL Protease Antibody - Interior 1	Novus Biologicals	NBP3-05697	WB, E
SARS-CoV-2 3CL Protease Antibody - Interior 2	Novus Biologicals	NBP3-05716	WB, E
SARS-CoV-2 3CL Protease Antibody - N-terminal	Novus Biologicals	NBP3-05717	WB, E
Human TMPRSS2 Antibody	Novus Biologicals	NBP2-97969	IHC

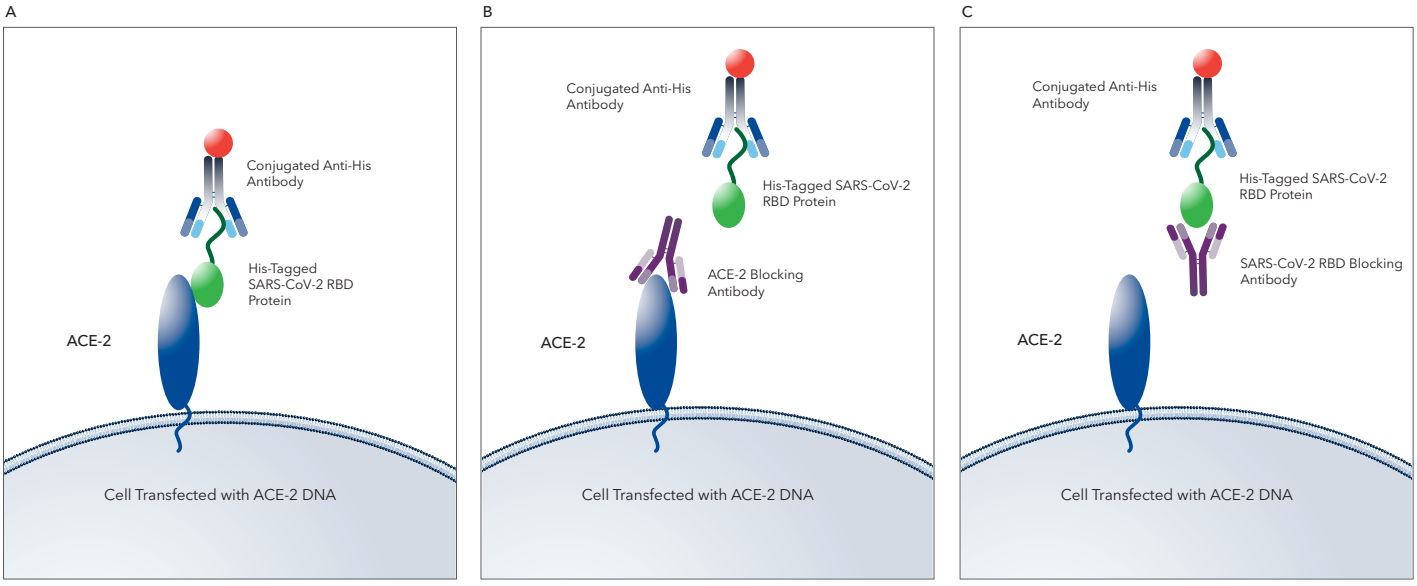
Human TMPRSS2 Antibody	Novus Biologicals	NBP1-20984	E, SW, WB
Human TMPRSS2 Antibody	Novus Biologicals	NBP2-38263	IHC, WB
Human TMPRSS2 Antibody	Novus Biologicals	NBP3-00492	IHC, WB

Application Key: **E** ELISA, **IHC** Immunohistochemistry, **SW** Simple Western, **WB** Western blot

SARS-COV-2 SPIKE RBD AND ACE-2 BLOCKING ANTIBODIES AND A FLOW CYTOMETRY-BASED *IN VITRO* BLOCKING ASSAY

The S protein on SARS-CoV-2 recognizes and binds to ACE-2 through its RBD. Thus, both proteins could be viable therapeutic targets. For example, blocking the interaction between ACE-2 and the S protein, with antibodies directed against either ACE-2 or the S Protein RBD has the potential to prevent SARS-CoV-2 infection. Since the development of COVID-19 therapeutics is encumbered by the fact that SARS-CoV-2 exhibits high pathogenicity and infectivity and needs to be handled under biosafety level 3 conditions, we developed a flow cytometry-based, *in vitro* assay for evaluating the effectiveness of antibodies and/or small molecules to block the binding of the S protein to ACE-2.

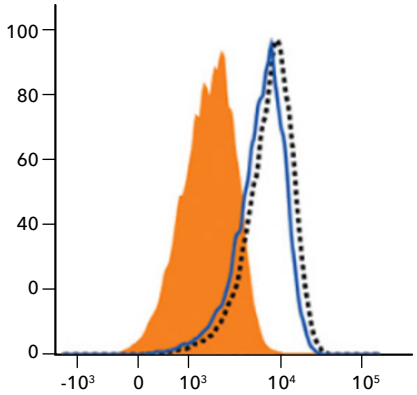
- Take advantage of our decades of bioassay experience and expertise developing blocking antibodies
- This surrogate assay does not require BSL-2 or BSL-3 biocontainment facilities
- Research antibodies that block SARS-CoV-2 spike protein, RBD protein or the ACE-2 receptor are available
- Custom recombinant antibody engineering options include LlaMABody™ Camelid single domain antibodies



Flow Cytometry *In Vitro* Blocking Assay Prinicple. (A) Cells are transfected with human ACE-2 DNA and express the receptor on their cell surface. When incubated with a recombinant SARS-CoV-2 Spike RBD His-tag Protein, the protein will bind to ACE-2. SARS-CoV-2 RBD protein binding is detected with an APC-conjugated His-Tag Antibody. **(B)** Incubating cells with an ACE-2 blocking antibody prior to the SARS-CoV-2 Spike RBD Protein allows the ACE-2 antibody to bind to the ACE-2 receptor, blocking SARS-CoV-2 Spike RBD protein binding. (C) Incubating SARS-CoV-2 Spike RBD protein with a SARS-CoV-2 Spike RBD blocking antibody prior to incubating with the cells allows the blocking antibody to bind to the SARS-CoV-2 Spike RBD protein and disrupt its interaction with ACE-2.

FLOW CYTOMETRY BLOCKING ASSAY TOOLS			
PRODUCT	BRAND	CATALOG #	ADDITIONAL INFORMATION
Recombinant SARS-CoV-2 Spike Protein RBD	R&D Systems	10499-CV	Fc tag, HEK293-derived
		10500-CV	His-tag, HEK293-derived
		10534-CV	His-tag, CHO-derived
		10523-CV	His-tag, Tn5-derived
		NBP2-90982	His-tag

PRODUCT	BRAND	CATALOG #	APPLICATIONS	ADDITIONAL INFORMATION
Goat Anti-Human ACE-2 Polyclonal Antibody	R&D Systems	AF933	B/N, FC, IHC, IP, SW, WB	Conjugates Available: FAB933-A, G, N, P, R, S, T, U, V
Mouse Anti-Human ACE-2 Monoclonal Antibody	Novus Biologicals	NBP2-80038	B/N, E, FA, FC, IHC, WB	Conjugates Available
SARS-CoV-1/2 Spike Llamabody Antibody	R&D Systems	LMAB10541	B/N, E	New camelid antibody
SARS-CoV-2 Spike RBD Antibody	R&D Systems	MAB105802	B/N, IHC	
SARS-CoV-2 Spike RBD Antibody	R&D Systems	MAB105801	B/N	



Blocking SARS-CoV-2 Binding to ACE-2 with a SARS-CoV-1/2 Spike RBD Llamabody. In a functional flow cytometry test, Recombinant SARS-CoV-2 Spike RBD His-tag Protein (Catalog # 10534-CV) binds to HEK293 human embryonic kidney cell line transfected with human ACE-2 (black dotted line). Protein binding was detected with an APC-conjugated Mouse Anti-His Tag Monoclonal Antibody (R&D Systems, Catalog # IC050A). Spike RBD binding to ACE-2 is completely blocked by an Anti-SARS-CoV-1/2 Spike RBD Llamabody Antibody (R&D Systems, Catalog # LMAB10541; orange histogram). A Mouse Anti-Human DC-SIGN/CD209 Monoclonal Antibody (R&D Systems, Catalog # MAB161) was used as an irrelevant control (blue line).

Application Key: **B/N** Blocking/Neutralization **E** ELISA **FA** Functional Assay **FC** Flow Cytometry **IHC** Immunohistochemistry **IP** Immunoprecipitation **SW** Simple Western **WB** Western Blot

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