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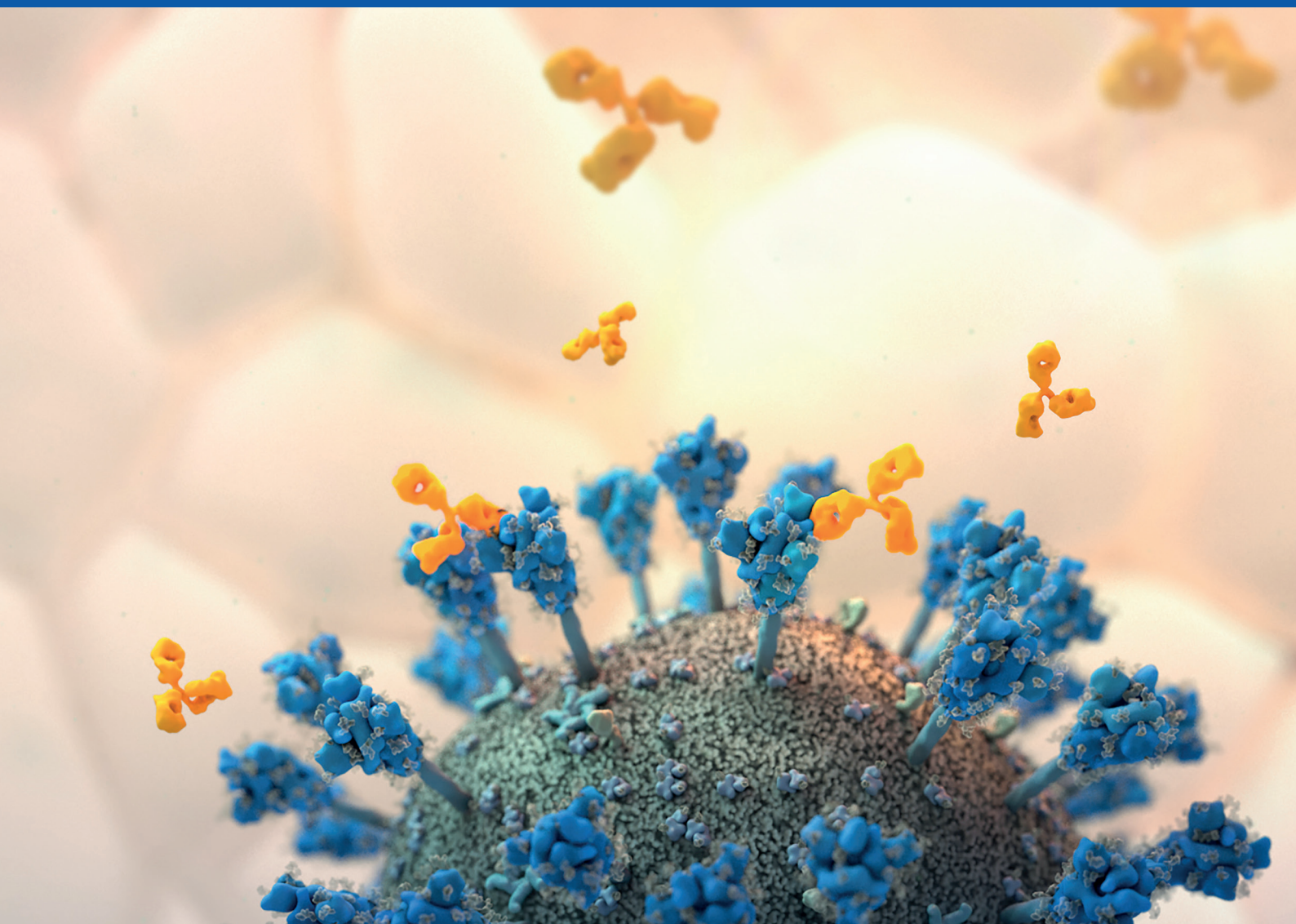
InVivo BioTech Services GmbH
a BRUKER company

SARS-CoV-2 Reagents



ABOUT SARS-COV-2

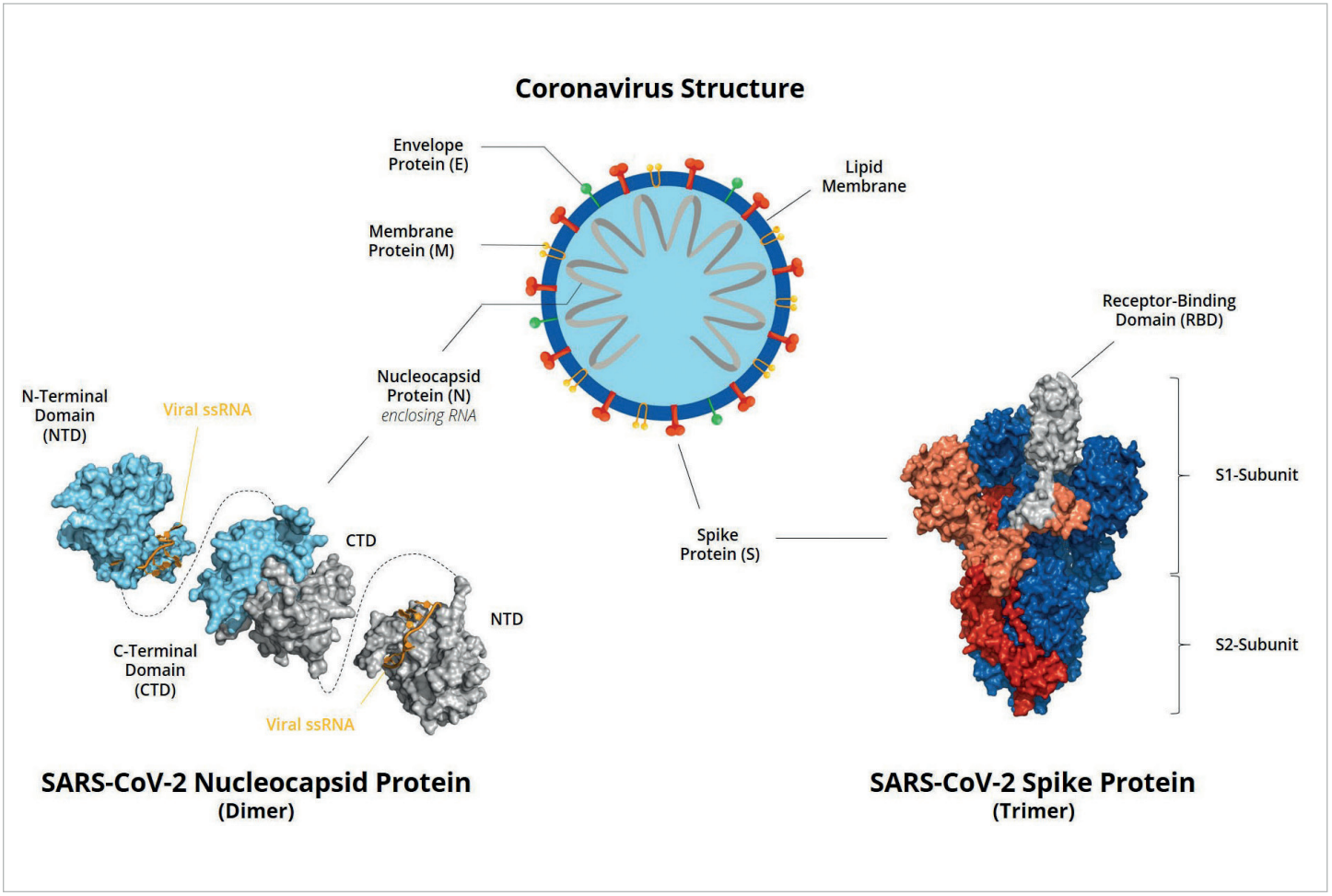
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), initially known as 2019 new coronavirus (2019-nCoV) belongs to the group of coronaviruses (CoV) and is the seventh coronavirus known to cause disease in humans. While some CoVs cause only mild, cold-like illnesses, an infection with MERS-CoV, SARS-CoV and SARS-CoV-2 can result in life-threatening disease. Illnesses caused by SARS-CoV-2, were designated Coronavirus Disease 2019 (COVID-19). Symptoms include fever, severe respiratory illness, and pneumonia.

SARS-CoV-2 has spread globally, causing a pandemic with millions of fatalities. To fight this pandemic, a variety of vaccines have been rolled out and billions of doses have been administered so far.
However, newly emerging variants of this virus that show an increased transmission rate and/or the ability to escape detection by the human immune system, raise the concern about further waves of infections.

Coronaviruses are enveloped RNA-viruses with a large genome (29.9 kb) that codes for diverse structural and functional proteins. Of these proteins, the Spike (S) protein and the Nucleocapsid (N) protein, are of special interest, as they represent useful targets for the development of highly-effective *in-vitro* diagnostic test kits and therapeutics.

The S-protein plays a crucial role in the infection process. It consists of two functional subunits: S1 and S2. While the S1-subunit enables binding of the virion to the ACE2 receptor on host cell membranes to initiate the infection process, the S2-subunit is responsible for the fusion of the virion on the cellular membranes.

The N-protein is the major structural component of the virion. It is involved in viral replication, assembly and immune regulation and plays key roles in the viral life cycle.

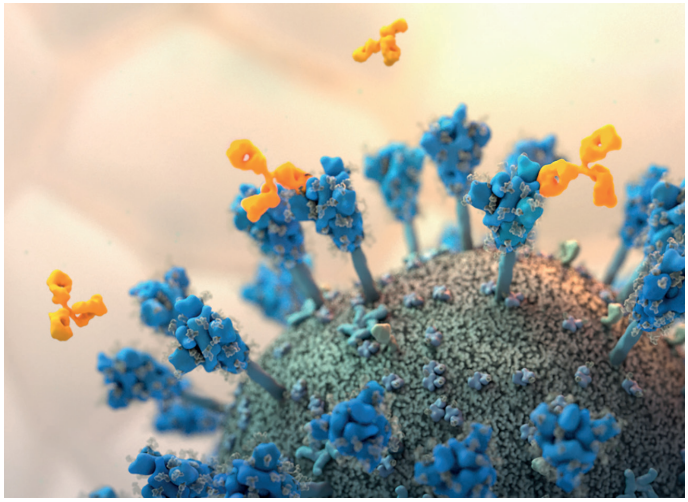


SARS-COV-2 ANTIGENS

To support the fight against the current pandemic, InVivo has developed a large set of reagents for COVID-19 research and manufacturing of highly sensitive *in-vitro* diagnostics test kits.

InVivo offers different recombinant versions of the SARS-CoV-2 S-protein that were produced in mammalian expression systems. Using InVivo's own platform **InVest,** high-yielding HEK-INV, cells were transiently transfected to produce a soluble version of the full-length Spike protein (Soluble Spike; according to Amanat *et al.* 2020), the two functional subunits S1 and S2, and the S1 receptor-binding domain (RBD).
To cater to long-term demand of bulk quantities in the gram scale for IVD kit manufacturing, InVivo used **stable CHO cell pools** to produce Soluble Spike protein and the S1-RBD.

All antigens were equipped with either a C-terminal poly-histidine or Fc-tag (see description) and purified via 2-step purification to obtain purity ≥ 90%.
Proper glycosylation was analyzed through intact mass analysis and protein activity was verified using an in-house



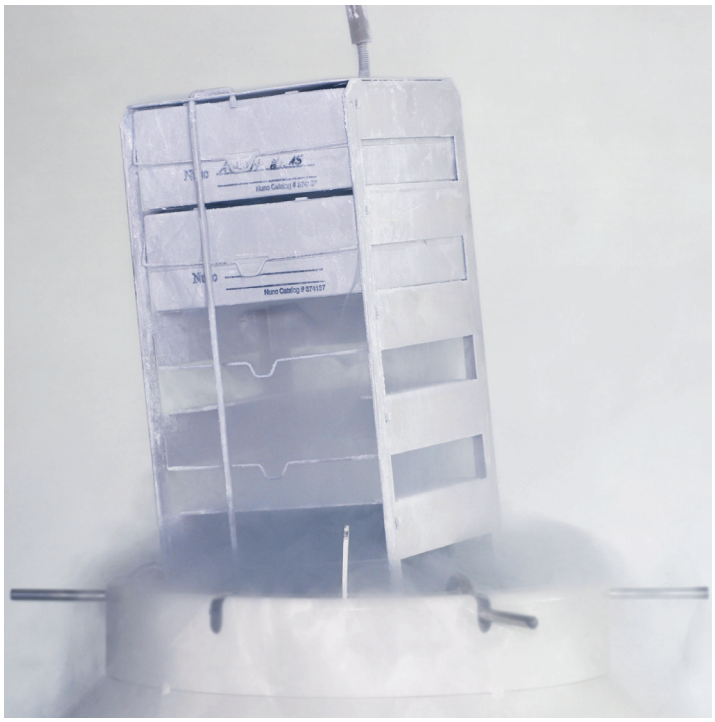
SARS-CoV-2 IgG ELISA setup.

Moreover, InVivo offers the SARS-CoV-2 Nucleocapsid protein (NP) from bacterial expression. The protein was purified via immobilized metal exchange chromatography (IMAC) using a C-terminal deca-histidine tag.

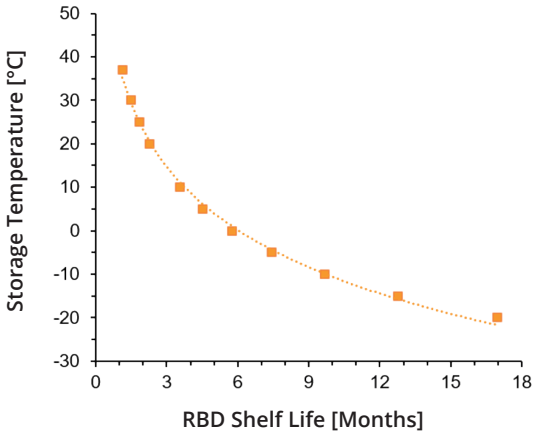
SARS-CoV-2 Antigens

Product-ID	Description
S1-RBD_CHO	Spike protein RBD (aa 319-541) with C-terminal hexa-his-tag
Soluble Spike_CHO	Soluble Spike protein (aa 14-1213) with mutated polybasic/ furin cleavage site, and K986P/ V987P for stabilization of the trimer. The C-terminal transmembrane domain (TMD) and endodomain were replaced by a thrombin cleavage and a trimerization site as well as a hexa-his-tag
S1-RBD_HEK	Spike protein RBD (aa 319-541) with C-terminal hexa-his-tag
S1-Subunit_HEK	Spike protein S1-subunit (aa 14-681) with C-terminal hexa-his-tag
S1-Subunit-Fc_HEK	Spike protein S1-subunit (aa 14-681) with C-terminal Fc-tag
S2-Subunit-Fc_HEK	Spike protein S2-subunit (aa 685-1213; excluding TMD and endodomain) with C-terminal Fc-tag
Soluble Spike_HEK	Soluble Spike protein (aa 14-1213) with mutated polybasic/ furin cleavage site, and mutations K986P/V987P for stabilization of the trimer. The C-terminal TMD and the endodomain were replaced by a thrombin cleavage and a trimerization site, as well as a hexa-his-tag
NP_E.coli	Nucleocapsid protein (aa 1-419) with C-terminal deca-his-tag

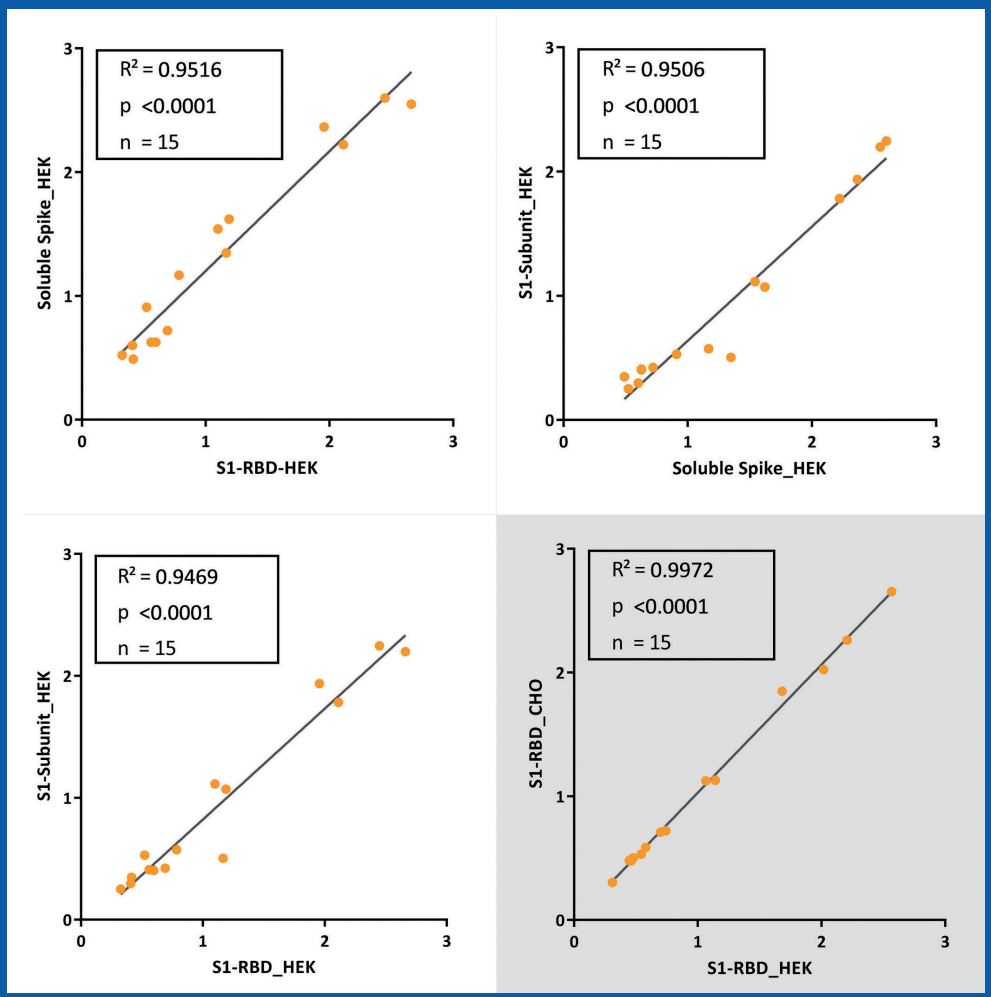
SARS-COV-2 ANTIGENS



Extrapolated stability of S1-RBD at different storage temperatures



For regular usage, InVivo recommends to store S1-RBD in the refrigerator (at 2-8°C). For long-term storage, aliquoted material should be stored in the freezer (-20°C).



Comparison of the binding activities of Soluble Spike protein, S1-subunit and S1-RBD produced from transient gene expression in HEK cells (white box) and comparison of the binding activies of S1-RBD produced via transient gene expression in HEK cells and from stable CHO cell pools (grey box).

An in-house IgG ELISA setup was used for the analysis of binding activities of the respective antigens.
Therefore, every antigen was directly coated to immunoassay plates and the binding activity of IgG antibodies obtained from serum of 15 former SARS-CoV-2 patients was analyzed.

SARS-COV-2 MUTANT ANTIGENS

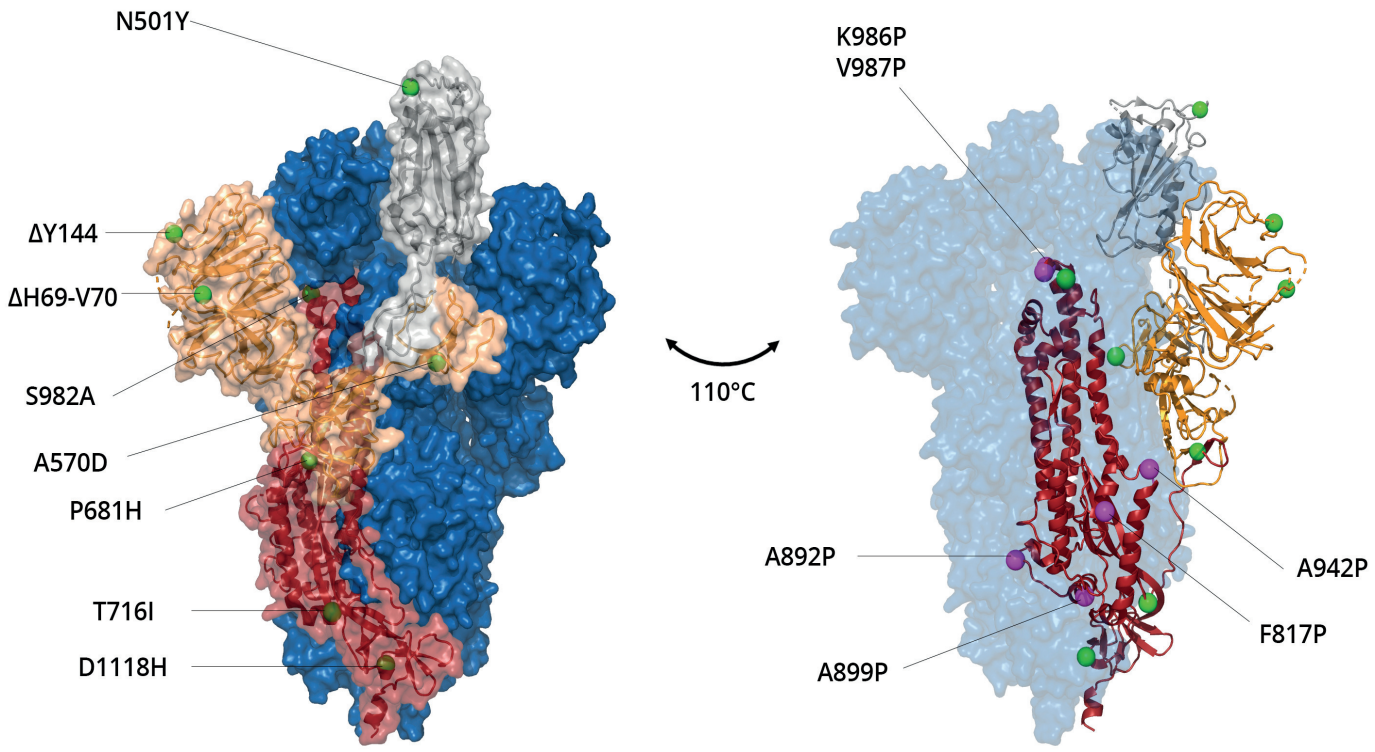
Throughout the COVID-19 pandemic, several genetic variants of SARS-CoV-2 have emerged worldwide, which were classified as so-called variants of concern (VOC) or variants of interest (VOI).
Among other things, there is evidence for an increased transmissibility of these virus variants and a significant reduction in neutralization through existing antibodies. Many mutations, such as amino acid substitutions and/or deletions, affect the S-protein and its RBD.

To investigate the influence of these mutations on the effectiveness of SARS-CoV-2 diagnostics, InVivo offers a series of mutant Spike antigens.
These include several RBD mutants, such as the **Alpha** variant (B.1.1.7) with or without additional immune-escape mutation E484K, the **Beta** variant (B.1.351). the **Gamma** variant (B.1.1.28.1 / P.1), the **Delta** variant (B.1.617.2) and the **Kappa** variant (B.1.617.1).

Furthermore, the full-length S-protein mutant of the **Alpha** variant is available, as well as a stabilized hexa-Pro mutant of the Alpha variant.

All antigens were produced under serum-free conditions in high-yielding HEK cells (**InVEST**). The antigens include a C-terminal poly-histidine-tag and were purified via IMAC and preparative SEC (for polishing) to obtain purity ≥ 90%. The protein activity was validated through an in-house SARS-CoV-2 IgG ELISA.

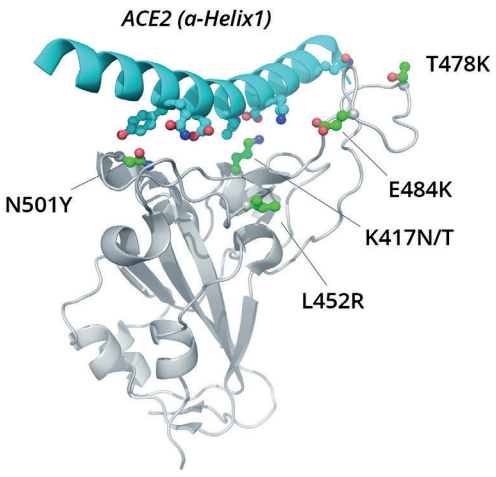
In case further variants of interest emerge, antigens from these strains can be made available within 8-12 weeks.



SARS-COV-2 MUTANT ANTIGENS

Overview of mutations that occur in the RBDs of different SARS-CoV-2 variants

	Alpha (B.1.1.7)	Alpha (B1.1.17) + E484K	Beta (B.1.351)	Gamma (P.1)	Kappa (B.1.617.1)	Delta (B.1.617.2)
K417T				X		
K417N			X			
L452R					X	X
E484K		X	X	X		
E484Q					X	
T478K						X
N501Y	X	X	X	X		



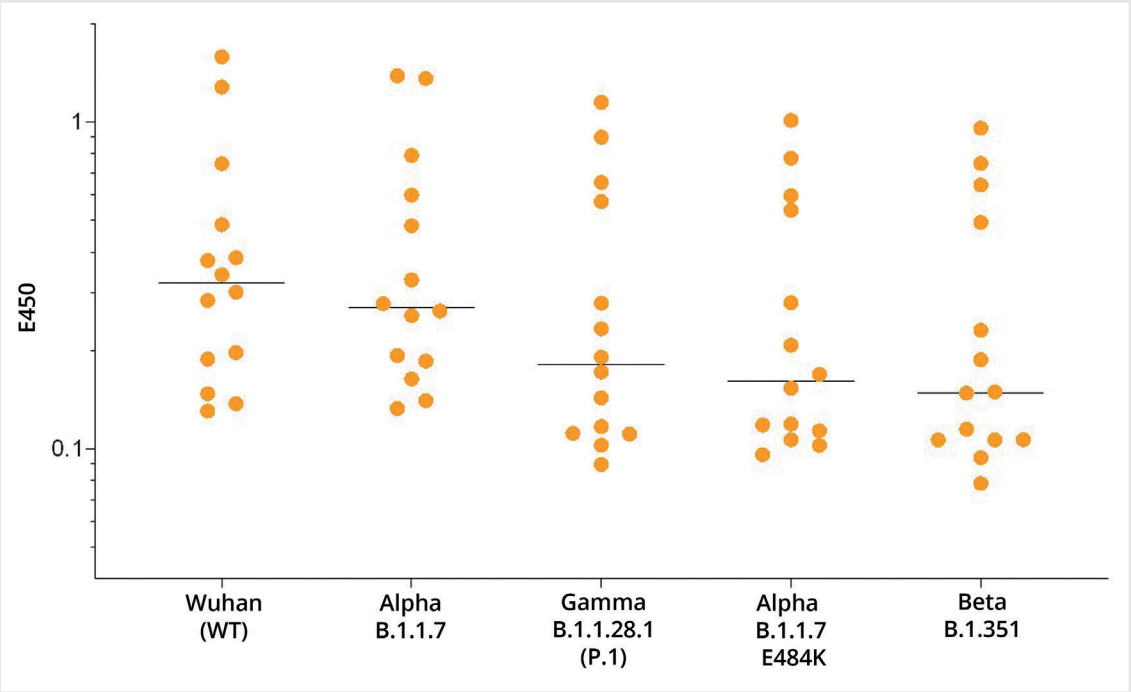
Spike S1-RBD
all mutants

S-protein and RBD variants

Product-ID	Description
Spike-Alpha (B.1.1.7)_HEK	S-protein of variant Alpha (B.1.1.7) Mutations HV 69-70 del, Y144 del, N501Y, A570D, P681H, T716I, S982A, D1118H; based on InVivo's "Soluble Spike" protein
Spike_HexaPro-Alpha (B.1.1.7)_HEK	S-protein of variant Alpha (B.1.1.7) Mutations HV 69-70 del, Y144 del, N501Y, A570D, P681H, T716I, S982A, D1118H; with additional mutations F817P, A892P, A899P, A942P, K986P, V987P (HexaPro) for stabilization of the protein, according to Hsieh *et al.* 2020; based on InVivo's "Soluble Spike" protein
S1-RBD-Alpha (B.1.1.7)_HEK	RBD of variant Alpha (B.1.1.7) Mutation N501Y; based on InVivo's RBD protein
S1-RBD-Alpha (B.1.1.7)+E484K_HEK	RBD of variant Alpha (B.1.1.7) Mutation N501Y and additional immune-escape mutation E484K; based on InVivo's RBD protein
S1-RBD-Beta (B.1.351)_HEK	RBD of variant Beta (B.1.351) Mutations K417N, E484K, N501Y; based on InVivo's RBD protein
S1-RBD-Gamma (B.1.1.28.1)_HEK	RBD of variant Gamma (B.1.1.28.1) Mutations K417T, E484K, N501Y; based on InVivo's RBD protein
S1-RBD-Kappa (B.1.617.1)_HEK	RBD of variant Kappa (B.1.617.1) Mutations L452R, E484Q; based on InVivo's RBD protein
S1-RBD-Delta (B.1.617.2)_HEK	RBD of variant Delta (B.1.617.2) Mutations L452R, T478K; based on InVivo's RBD protein

SARS-COV-2 MUTANT ANTIGENS

Binding activity comparison of recombinant wildtype (Wuhan) and mutant RBDs to IgG antibodies of recovered SARS-CoV-2 patients



RBDs obtained from transient gene expression in HEK cells were tested as solid phase-bound capture antigen at 2 µg/ mL in an in-house SARS-CoV-2 IgG ELISA setup using serum samples from fourteen SARS-CoV-2 positive patients (obtained before October 2020). The line indicates the median of the absorbance values.

Didn't find what you were looking for?

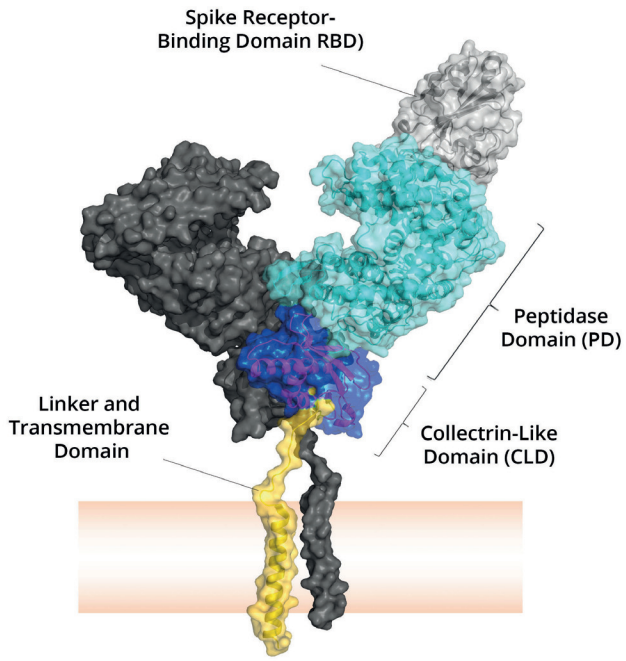
InVivo also offers custom solutions for the production of your specific antigen of interest. Depending on your needs, recombinant proteins can be made available from different mammalian expression systems (HEK or CHO) and in variable quantities. From small scale (milligram level) for research purposes up to industrial scale (gram level) for IVD kit manufacturing. Reach out to learn more about our services and obtain expert support for realizing your custom project.

OTHER RECOMBINANT PROTEINS

Angiotensin-converting enzyme 2 (ACE2) is a zinc-containing metalloenzyme, which regulates blood pressure through proteolysis of Angiotensin II. ACE2 also serves as an entry point into the cells for different coronaviruses, including SARS-CoV-2.

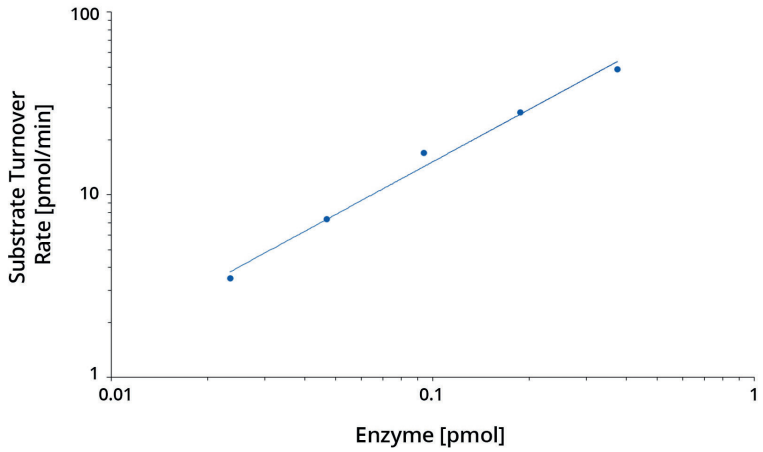
Using the Spike protein RBD which protrudes from the viral membrane surface, the virus binds to the peptidase domain (PD) of ACE2, thereby initiating the infection process. The collectrin-like domain (CLD) is responsible for the homo-dimerization of ACE2.

InVivo offers a recombinant form of the full-length ACE2 ectodomain, as well as the ACE2-PD. Both proteins were produced under serum-free conditions in HEK-INV cells and purified by IMAC using a C-terminal hexa-histidine-tag.



ACE2 (Dimer) with Spike-RBD

Substrate turnover rate of ACE2-PD_HEK over enzyme amount



Substrate turnover rates (= enzyme activity) of different amounts of ACE2-PD_HEK.

Mca-YVADAPK-Dnp was used as substrate and 7-Methoxycoumarin-4-acetic acid as calibrator. The reaction was performed at 37°C in a reaction buffer containing 1M NaCl and 10 µM $ZnCl_2$ at pH 7.5. The excitation wavelength was 340 nm and emission was measured at 415 nm.

Recombinant ACE2 Proteins

Product-ID	Description
ACE2_HEK	Recombinant ACE2 (1-740) with native signal peptide and the entire extracellular domain (including PD and CLD); with C-terminal hexa-his-tag
ACE2-PD_HEK	Recombinant ACE2 (1–615) with native signal peptide and PD (excluding CLD); with C-terminal hexa-his-tag

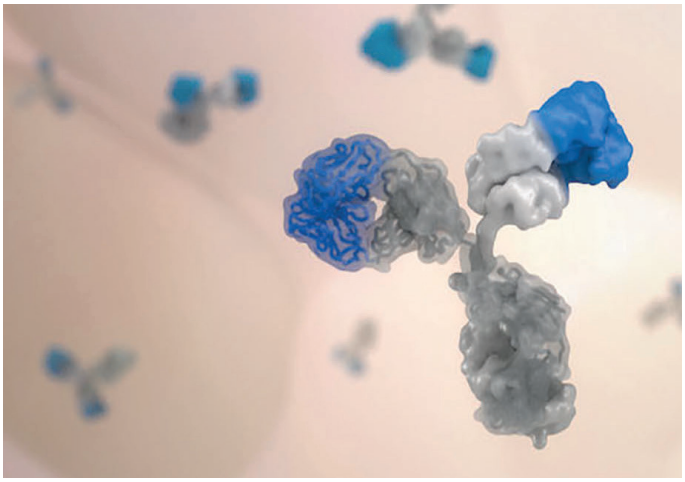
SARS-COV-2 ANTIBODIES

For industry-scale manufacturing of SARS-CoV-2 detection kits (ELISA, Lateral Flow and more), InVivo provides a set of different monoclonal IgG antibodies from mouse hybridoma, which selectively target either the SARS-CoV-2 S-protein or the N-protein.

Antibodies were generated by mouse immunization with the recombinant full-length Soluble Spike protein, its RBD or the N-protein (all antigens manufactured by InVivo).

The antibodies were produced from hybridoma under serum-free conditions and purified via Protein-A affinity chromatography to a purity ≥ 90%.

All antibodies were intensively tested by ELISA; as indicated in the table below, certain antibodies are suitable for Sandwich-ELISA applications or neutralization assays.

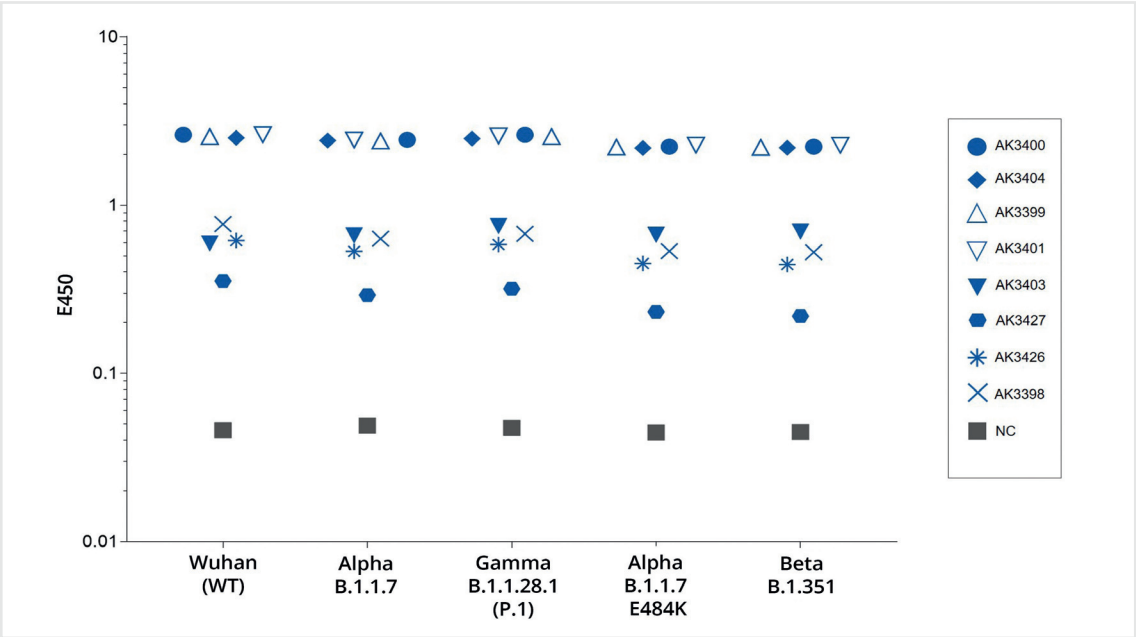


Anti-Spike Antibodies

Product-ID	Description	Specificity	Application	Neutralizing
AK3398	Anti-Spike (RBD) Antibody	RBD	ELISA	X
AK3399	Anti-Spike (RBD) Antibody	RBD	ELISA	
AK3400	Anti-Spike (RBD) Antibody	RBD	ELISA	X
AK3401	Anti-Spike (RBD) Antibody	RBD	ELISA	
AK3402	Anti-Spike (RBD) Antibody	RBD	ELISA	X
AK3403	Anti-Spike (RBD) Antibody	RBD	ELISA, Sandwich-ELISA	
AK3404	Anti-Spike (RBD) Antibody	RBD	ELISA, Sandwich-ELISA	X
AK3422	Anti-Spike (S1) Antibody	S1	ELISA	
AK3423	Anti-Spike (S1) Antibody	S1	ELISA, Sandwich-ELISA	
AK3424	Anti-Spike (S1) Antibody	S1	ELISA	
AK3426	Anti-Spike (RBD) Antibody	RBD	ELISA	
AK3427	Anti-Spike (RBD) Antibody	RBD	ELISA, Sandwich-ELISA	
AK3429	Anti-Spike (S1) Antibody	S1	ELISA, Sandwich-ELISA	

SARS-COV-2 ANTIBODIES

Binding activity of anti-Spike antibodies to S1-RBDs from different SARS-CoV-2 variants



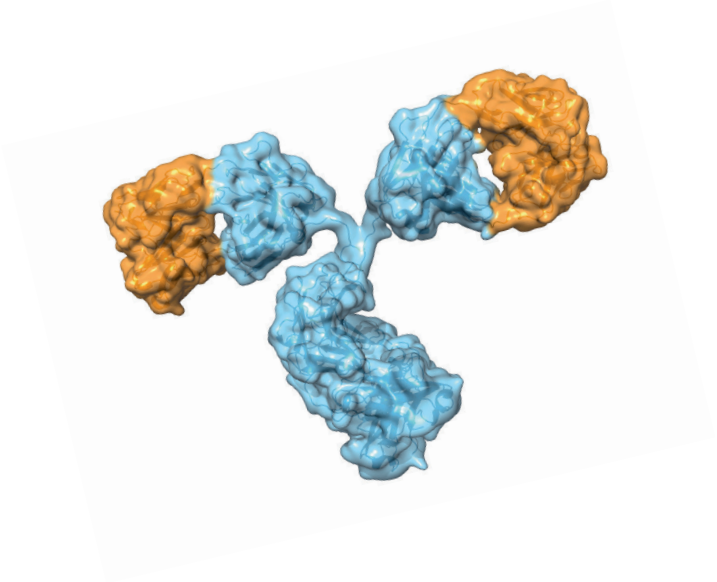
Analysis of the binding activity of InVivo's anti-Spike-antibodies to the S1-RBD from the wildtype strain (Wuhan; WT) and from different SARS-CoV-2 variants (Alpha B.1.1.7 w/o immune-escape mutation E484K, Gamma B.1.1.28.1 and Beta B.1.351).

Bound antibodies were detected using anti-mouse-IgG antibodies conjugated to HRP in an in-house SARS-CoV-2 IgG ELISA setup. The negative control is a monoclonal mouse hybridoma antibody which does not bind to the S1-RBD of SARS-CoV-2.

Anti-Nucleocapsid Antibodies

Product-ID	Description	Specificity	Application
AK3432	Anti-NP Antibody	NP	ELISA, Sandwich-ELISA
AK3433.1	Anti-NP Antibody	NP	ELISA, Sandwich-ELISA
AK3454	Anti-NP Antibody	NP	ELISA
AK3455	Anti-NP Antibody	NP	ELISA
AK3456	Anti-NP Antibody	NP	ELISA
AK3457	Anti-NP Antibody	NP	ELISA
AK3458	Anti-NP Antibody	NP	ELISA
AK3459	Anti-NP Antibody	NP	ELISA

SARS-COV-2 ANTIBODIES

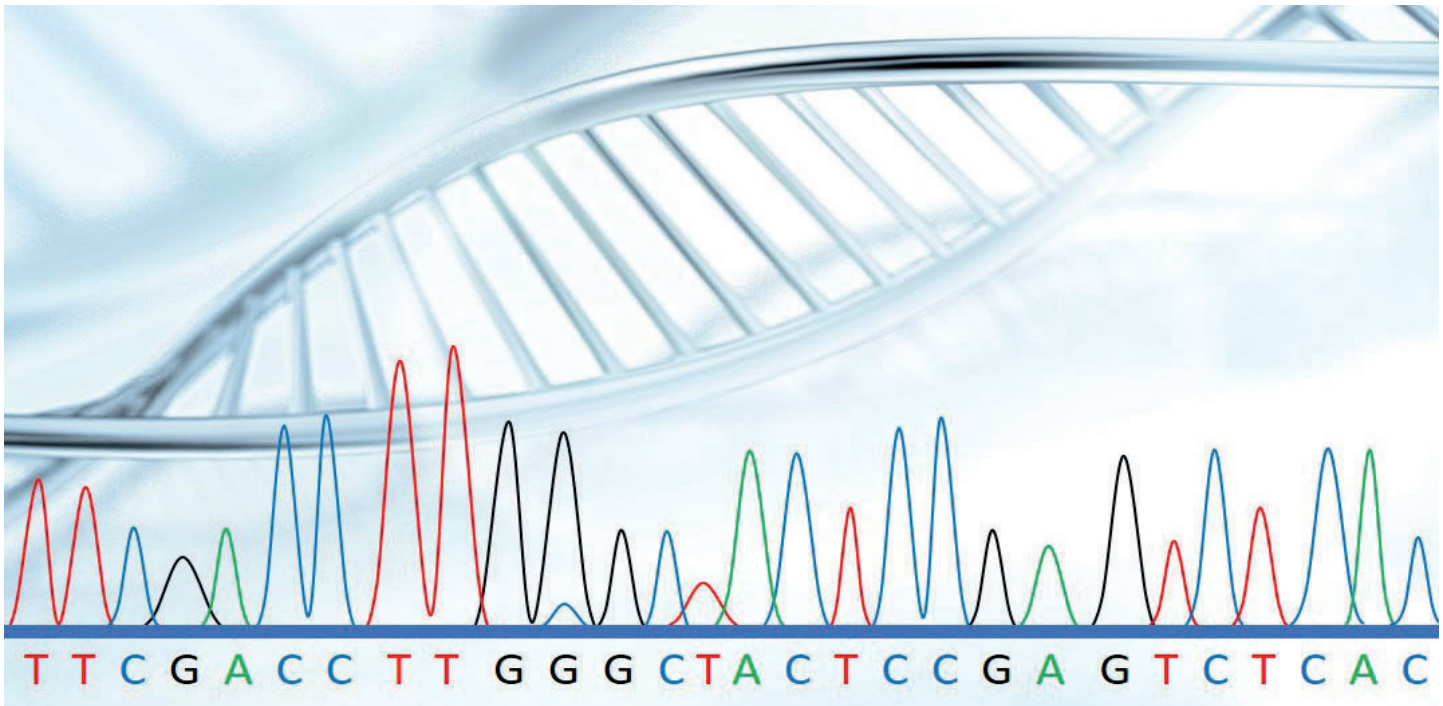


In addition to monoclonal antibodies from mouse hybridoma, InVivo also offers recombinant, chimeric antibodies containing mouse variable domains (V_L+V_H) fused with human IgG1 (C_H) and kappa (C_L) constant domains.

The recombinant antibodies were produced under serum-free conditions in high-yielding HEK cells and purified via Protein-A affinity chromatography to obtain purity ≥ 90%.

Recombinant Anti-Spike Antibodies

Product-ID	Description	Specificity
RP_SZ_824	Anti-Spike (RBD) rec Antibody The variable domain sequences correspond to those of murine antibodies AK3399 / AK3401 and are fused with human IgG1 (C_H) and kappa (C_L) constant domains.	RBD
RP_SZ_827	Anti-Spike (S1) rec Antibody The variable domain sequences correspond to those of murine antibodies AK3422 / AK3424 and are fused with human IgG1 (C_H) and kappa (C_L) constant domains.	S1



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