InVivo BioTech Services GmbH
a BRUKER company

PRODUCT INFORMATION

Anti-Spike (RBD) Antibody_AK3400

Description:

InVivo offers a series of different monoclonal antibodies for the detection of the SARS-CoV-2 Spike protein. All antibodies come from hybridoma cell lines that were generated by immunization with the recombinant full-length Spike protein (S) or the receptor-binding domain (RBD).

This antibody has been validated by ELISA and is specifically directed against an epitope that is located on the RBD. Additionally, ELISAs with directly coated antigens proved that this antibody also binds to the Alpha variant of the Spike protein and different RBD variants (Alpha, Alpha + E484K, Beta and Gamma).

This antibody was shown to neutralize the SARS-CoV-2 wildtype (Wuhan), as well as the Alpha and the Beta variant in cell culture. No other variants were tested.

All antibodies are produced exclusively under serum-free conditions from hybridoma and purified through one-step purification with Protein-A affinity chromatography.

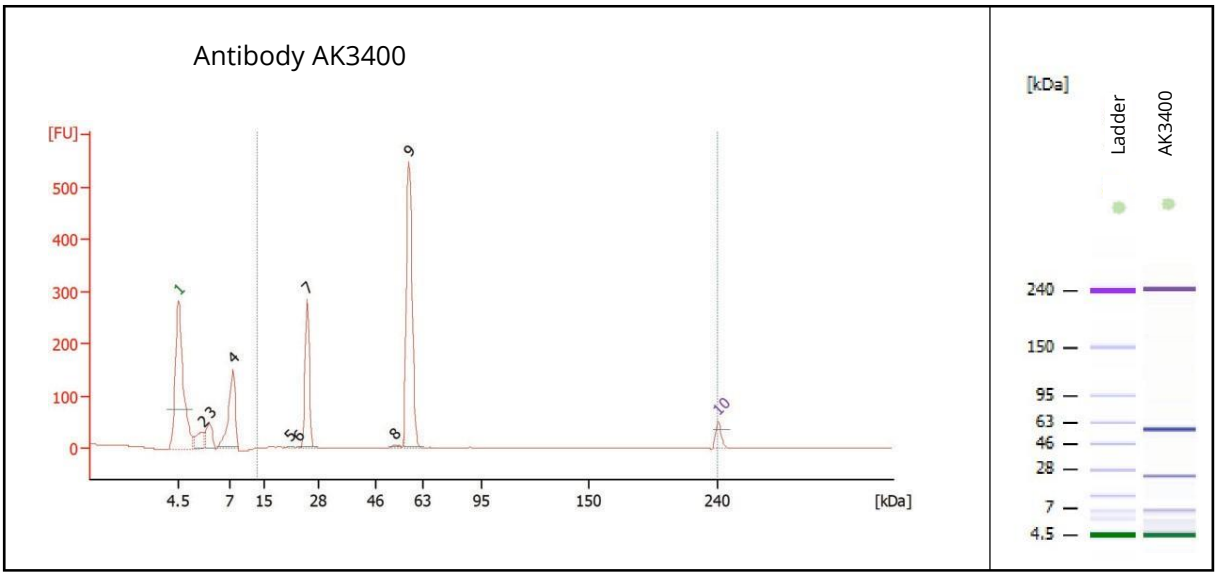
Product-ID:	AK3400
Host:	Mouse
Clonality:	Monoclonal
Isotype:	IgG
Subclass:	mIgG2a/bκ
Formulation:	Liquid, PBS, pH 7.4, 0.2 µm sterile filtered
Concentration:	≥ 0.5 mg/ mL
Purity:	≥ 90% (via analytical CGE under reducing conditions)
Conjugate:	Unconjugated
EC_{50} RBD[1]:	0.054 nM
EC_{50} S1[1]:	1.159 nM
EC_{50} S[1]:	0.374 nM
Specificity:	S (tested for: Wuhan and Alpha)
	RBD (tested for: Wuhan, Alpha, Alpha+E484K, Beta and Gamma)
Neutralization:	yes
IC_{50} SARS-CoV-2 Wuhan[2]:	0.26 +/- 0.04 µg/ mL
IC_{50} SARS-CoV-2 Alpha[2]:	0.12 +/- 0.01 µg/ mL
IC_{50} SARS-CoV-2 Beta[2]:	0.12 +/- 0.002 µg/ mL

The product is for research use or for further manufacturing only.

[1] EC_{50} values for 20 nM of coated antigen (Wuhan origin)

[2] Mean of two individual in-cell neutralization assays performed with Vera6 cells by our cooperation partner Dr. Valeria Falcone from the University Hospital Freiburg (Institute of Virology)

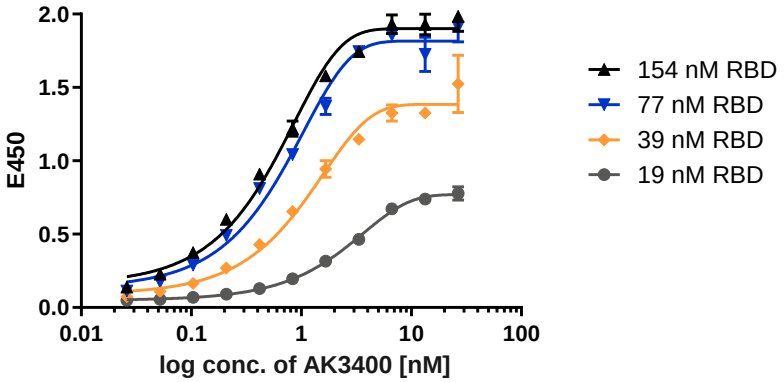
Purity (analytical CGE, under reducing conditions):



Peak	Size [kDa]	% of Total	Peak Identification
1	4.5	0.0	Lower Marker
2	5.7	0.0	System Peak
3	6.0	0.0	System Peak
4	7.9	0.0	System Peak
5	21.0	0.4	unknown
6	23.2	0.2	unknown
7	25.3	Peak Value 1	AK3400 Light Chain
8	52.9	0.9	unknown
9	57.9	Peak Value 2	AK3400 Heavy Chain
10	240.0	0.0	Upper Marker

Summation of peak values 1 and 2 results in a purity of ≥ 90%

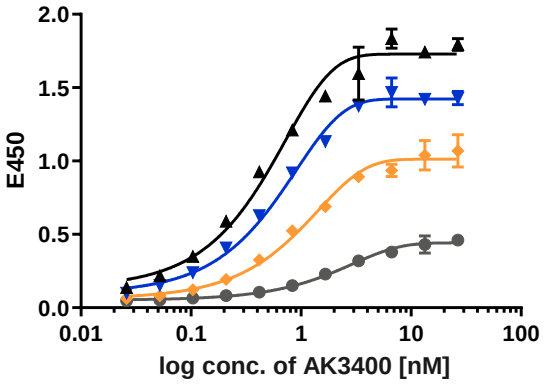
Protein Activity (ELISA):



For ELISA analyses immunoassay plates were coated with different concentrations of SARS-CoV-2 RBD (19, 39, 77 and 154 nM). Antibody AK3400 was applied at concentrations ranging from 26.67 nM to 0.026 nM.

Bound antibody AK3400 was detected using Goat Anti-Mouse-IgG-HRP (10 ng/mL) and TMB. Determined EC_{50} of antibody AK3400 for 20 nM RBD is 0.054 nM.

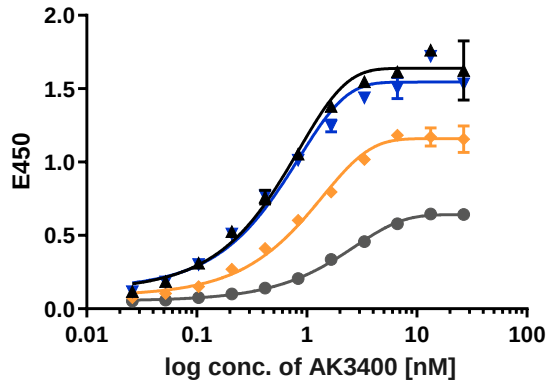
	154 nM RBD	77 nM RBD	39 nM RBD	19 nM RBD
EC_{50} [nM]	0.5508	0.6666	1.152	2.748



	84 nM S1	42 nM S1	21 nM S1	10 nM S1
EC_{50} [nM]	0.4551	0.5911	1.014	2.272

For ELISA analyses immunoassay plates were coated with different concentrations of SARS-CoV-2 S1 (10, 21, 42 and 84 nM). Antibody AK3400 was applied at concentrations ranging from 26.67 nM to 0.026 nM.

Bound antibody AK3400 was detected using Goat Anti-Mouse-IgG-HRP (10 ng/mL) and TMB. Determined EC_{50} of antibody AK3400 for 20 nM S1 is 1.159 nM.

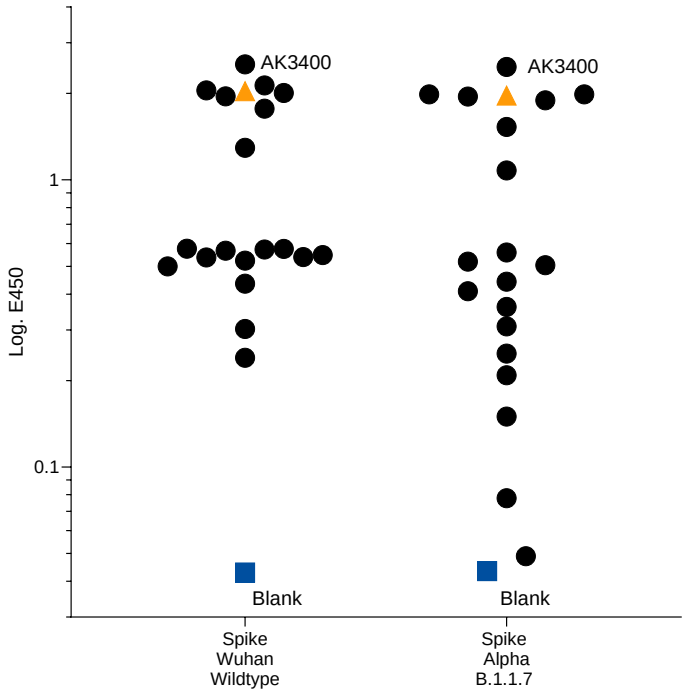


	29 nM S	14 nM S	7 nM S	3.6 nM S
EC_{50} [nM]	0.5491	0.5241	0.9229	1.992

For ELISA analyses immunoassay plates were coated with different concentrations of SARS-CoV-2 Spike protein (3.6, 7, 14 and 29 nM). Antibody AK3400 was applied at concentrations ranging from 26.67 nM to 0.026 nM.

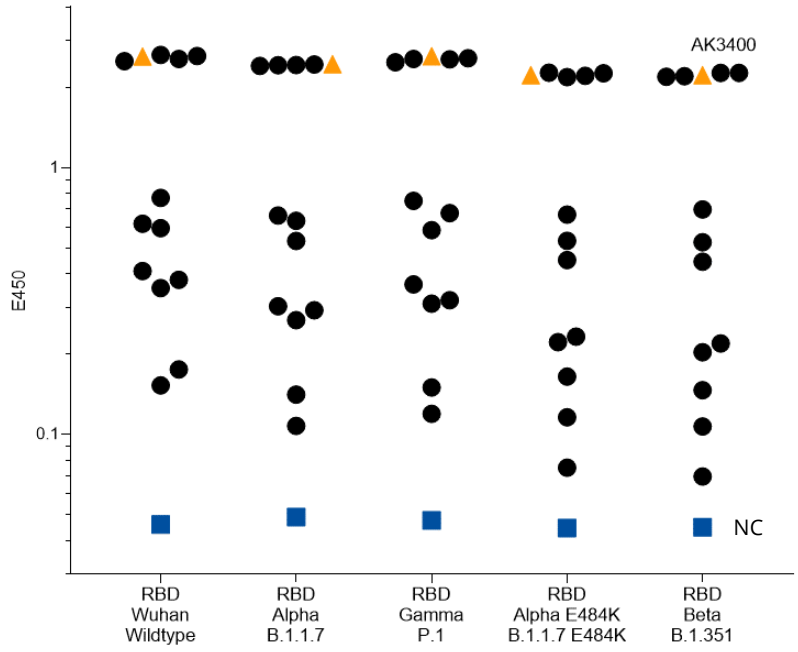
Bound antibody AK3400 was detected using Goat Anti-Mouse-IgG-HRP (10 ng/mL) and TMB. Determined EC_{50} of antibody AK3400 for 20 nM Spike protein is 0.374 nM.

Binding activity to SARS-CoV-2 Spike protein and receptor-binding domain variants (ELISA):



Binding activity of AK3400 to coated SARS-COV-2 Spike protein (Wuhan origin vs. Alpha) in comparison to 19 other monoclonal mouse hybridoma antibodies targeting the Spike protein.

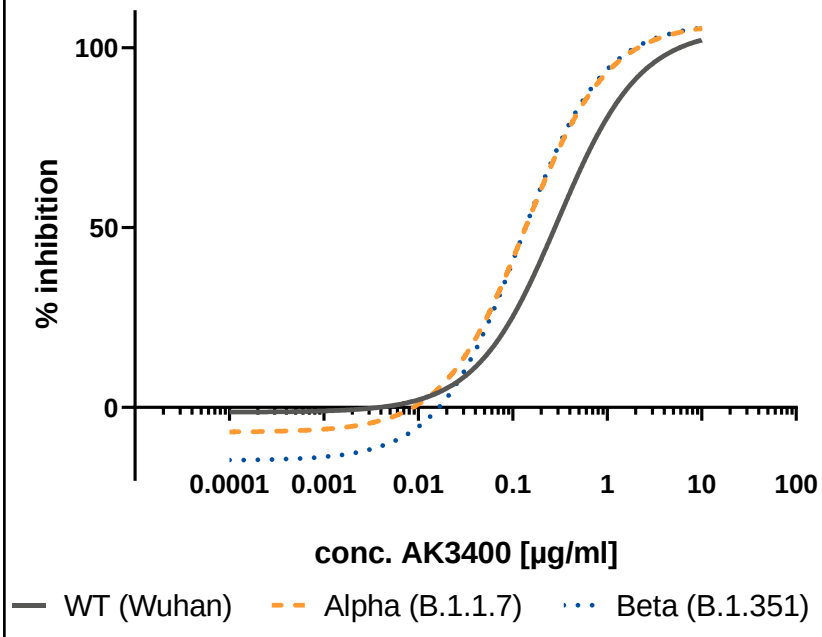
Bound antibodies were detected using anti- mouse-IgG antibodies conjugated to HRP in an in-house SARS-CoV-2 IgG ELISA. For the blank signal, no mouse-antibody was added.



Binding activity of AK3400 to different coated SARS-COV-2 RBD variants (WT, Alpha, Gamma, Alpha E484K and Beta) in comparison to 12 other monoclonal mouse hybridoma antibodies targeting the RBD and a negative control antibody (NC).

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

Neutralization Assay:



Dose-dependent inhibition of SARS-CoV-2 infection with wildtype and common variants (Alpha and Beta) by AK3400 measured in an in-cell ELISA assay with Vero6 cells.

As an example, the results of one out of two individual assays are depicted.