AI334, AQ806, AR222, AR249, AS274, AS702, AS708, RB590, RB591 and RB596 antibodies recognize the spike S protein from SARS-CoV-2 by western blot

Anna Marchetti[1], Frederic Zenhausern[2,3]

[1] *Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*
[2] *Center for Applied NanoBioscience and Medicine, The University of Arizona, Phoenix, AZ 85004, USA*
[3] *School of Pharmaceutical Sciences, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*

Abstract
The recombinant antibodies AI334, AQ806, AR222, AR249, AS274, AS702, AS708, RB590, RB591 and RB596 detect by western blot the spike S protein from SARS-CoV-2.

Introduction
The spike (S) glycoprotein mediates attachment of coronaviruses to the host ACE2 receptor and fusion with the host cell membrane (Yan *et al.*, 2020). Ten recombinant antibodies (AI334, AQ806, AR222, AR249, AS274, AS702, AS708, RB590, RB591 and RB596) successfully detect by western blot the spike S protein from SARS-CoV-2 (UniProt P0DTC2) expressed in Vero-B4 cells.

Materials & Methods
Antibodies: ABCD_AI334, ABCD_AQ806, ABCD_AR222, ABCD_AR249, ABCD_AS274, ABCD_AS702, ABCD_AS708, ABCD_RB590, ABCD_RB591 and ABCD_RB596 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a mouse IgG2A Fc. The synthesized scFv or VHH sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (see Table 1 for clone names and references). HEK 293T suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the scFv-Fc or VHH-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Table 1: Clone number, epitope, reference and production yields for the antibodies used in this study.

ABCD	Clone	Epitope	Reference	Yield (mg/L)
AI334	CR3022	S1	ter Meulen et al., 2006	50
AQ806	VHH-72	S1/RBD	Wrapp et al., 2020	50
AR222	Sb#14	S1/RBD	Walter *et al.*, 2020	60
AR249	Sb#45	S1/RBD	Walter *et al.*, 2020	100
AS274	H4	S1/RBD	Wu *et al.*, 2020	20
AS702	CV24	S1	Seydoux *et al.*, 2020	20
AS708	CV30	S1/RBD	Seydoux *et al.*, 2020	20
RB590	RB590	Cytosolic	this work	50
RB591	RB591	Cytosolic	this work	120
RB596	RB596	S2	Farrera-Soler *et al.*, 2020	100

Antigen: Vero-B4 adherent cells (growing in DMEM, Gibco 31966021, supplemented with 10% FBS), transiently transfected 2 days before the experiment with a vector coding for the full-length SARS-CoV-2 S protein (BEI Resources, NR-52310, pCAGGS vector containing the full-length SARS-CoV-2/Wuhan-Hu-1 S glycoprotein coding sequence), were used to detect the full-length S protein. Non-transfected cells were used as a negative control.

Protocol: $5x10^6$ cells were pelleted and lysed for 15 min in 100 µL of ice-cold lysis buffer (25 mM Tris-HCl pH 7.4 + 0.5 % Triton X-100 + 120 mM NaCl) containing protease inhibitors. Lysate was centrifuged 15 min, 10'000 g at 4 °C to remove nuclei. One volume of reduced sample buffer was added to the lysate (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol) and boiled for 15 min at 95 ºC. 10 µL of each sample ($2.5x10^5$ cells) was migrated (150 V, 45 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen IB23001). The membranes were blocked during 60 min in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed once for 15 minutes in PBS + 0.1% (v/v) Tween20 (PBS-Tween). The membranes were then incubated overnight at RT with the anti-S antibodies (final concentration 5 mg/L in PBS-Tween). The membranes were then washed three times (15+15+10 min) in PBS-Tween, incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad, 170-6516, dilution 1:3000) and washed three times (15 min) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results
AI334, AQ806, AR222, AR249, AS274, AS702, AS708, RB590, RB591 and RB596 antibodies specifically recognize the S protein in Vero-B4 transfected cells (Fig. 1). The ~180 kDa and the ~90 kDa bands correspond to the full-length and cleaved S proteins; higher molecular weight bands (>250 kDa) correspond to oligomerized (dimeric or trimeric) S proteins (Ou *et al.*, 2020). The AI334 antibody also recognizes a non-specific band (~250 kDa) on non-transfected cells (Fig. 1).

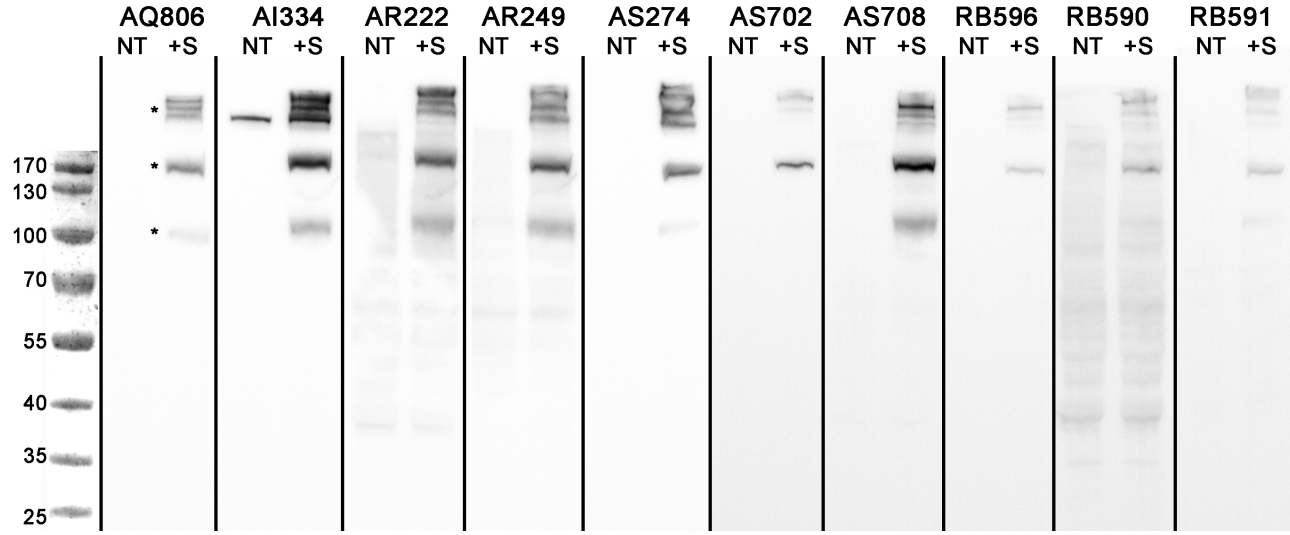

Fig. 1. Antibodies AI334, AQ806, AR222, AR249, AS274, AS702, AS708, RB590, RB591 and RB596 specifically recognize the spike S protein from SARS-CoV-2 (the asterisks (*) denote the main forms of the protein: cleaved, full-length, and oligomerized; shown only for AQ806).

References

Farrera-Soler L, Daguer JP, Sofia Badiola B, Winssinger N. The RB596 antibody recognizes a linear epitope from the spike S protein from SARS-CoV-2. Antibody Reports, 2020, 3:e232. doi:10.22450/ journals/abrep.2020.e232

Ou X, Liu Y, Lei X, *et al*. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020; 11:1620. PMID: 32221306

Seydoux E, Homad LJ, MacCamy AJ, *et al*. Analysis of a SARS-CoV-2-infected individual reveals development of potent neutralizing antibodies with limited somatic mutation. Immunity 2020; 53:1-8. PMID: 32561270.

ter Meulen J, van den Brink EN, Poon LL, *et al*. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. PLoS Med. 2006; 3:e237. PMID:16796401

Walter JD, Hutter CAJ, Zimmermann I, *et al*. Sybodies targeting the SARS-CoV-2 receptor-binding domain. Preprint. bioRxiv 2020; 2020.04.16.045419. doi:10.1101/2020.04.16.045419

Wrapp D, De Vlieger D, Corbett KS, *et al*. Structural basis for potent neutralization of betacoronaviruses by single-domain camelid antibodies. Cell 2020; 181:1004-1015. PMID:32375025

Wu Y, Wang F, Shen C, *et al.* A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science 2020; 368:1274-1278. PMID:32404477

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020; 367:1444-1448. PMID:32132184

Acknowledgments

This work was co-sponsored by NASA TRISH contract #NNX16A069A/CAT0001. The following reagent was obtained through BEI Resources, NIAID, NIH: Vector pCAGGS containing the SARS-related Coronavirus 2, Wuhan-Hu-1 Spike glycoprotein gene, NR-52310.

Conflict of interest

The authors declare no conflict of interest.