AS739, AT693, AU197 and AU734 antibodies label the spike S protein from SARS-CoV-2 by western blot


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Abstract
The recombinant antibodies AS739, AT693, AU197 and AU734 detect by western blot the spike S protein from SARS-CoV-2.

Introduction
The spike S glycoprotein (UniProt P0DTC2) mediates the attachment of coronaviruses to the host ACE2 receptor (through the Receptor-Binding Domain [RBD] in the S1 subunit) and fusion with the host cell membrane (through the S2 subunit) (Yan et al., 2020). Five recombinant antibodies recognizing the S1 domain of the S protein from SARS-CoV-2 (AS739, AT693, AU197, AU734 and AU753) were tested for their ability to recognize the S protein by western blot. Four antibodies (AS739, AT693, AU197 and AU734) detected the S protein from SARS-CoV-2; one (AU753) did not.

Materials & Methods
Antibodies: ABCD_AS739, ABCD_AT693, ABCD_AU197, ABCD_AU734 and ABCD_AU753 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 rabbit IgG Fc. The synthesized scFv 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). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Antigen: Vero-B4 adherent cells (growing in DMEM, Gibco 11960044, supplemented with 10% FBS) were transiently transfected 48 hours 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). Non-transfected cells were used as a negative control.

Protocol: 5x10^5 transfected Vero-B4 cells were pelleted and lysed in PBS containing 0.5% (v/v) Triton X-100. Nuclei were pelleted by centrifugation (5 min at 12'000 g) and the supernatant was recovered and mixed with reducing or non-reducing sample buffer (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% (w/v) β-mercaptoethanol). Each sample was migrated (200 V, 30 min) in a 4-20% acrylamide gel (SurePAGE Bis-Tris, Genscript M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 10 min (iBlot gel transfer device, Invitrogen IB1001EU). The membranes were blocked 2 h in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk and washed three times for 5 min in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with the recombinant anti-S antibodies (dilution 1:10 in PBS-Tween-milk) for 30 min at room temperature and washed three times for 5 min. The membranes were then incubated for 30 min with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich A8275, dilution 1:3000 in PBS-Tween-milk) and washed 5 times for 5 min in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging system (Syngene).

Results
Antibodies AS739, AT693, AU197 and AU734 recognized by western blot the SARS-CoV-2 spike S protein expressed in Vero-B4 transfected cells and migrated in non-reducing conditions (Fig. 1). No signal was detected in non-transfected cells, or in reducing conditions (Fig. 1). AU753 did not recognize the S protein; this is possibly due to the fact that this antibody is poorly produced. Three bands were observed with the antibodies AS739 and AU197: (i) a ~120 kDa band, presumably corresponding to the full-length spike protein; (ii) a higher molecular weight band, likely a spike oligomer; and (iii) a smaller band at ~70 kDa, presumably corresponding to the processed S1 subunit of the spike protein. Antibodies AT693 and AU734 only recognized the high-molecular form of the protein. A weak signal was obtained with the antibody AT693.
References

Conflict of interest
The authors declare no conflict of interest.

Fig. 1. Specific binding of AS739, AU197, AU734 and AT693 antibodies to the SARS-CoV-2 S protein in Vero-B4 transfected (T) cells in non-reducing (NR) conditions, but not in reducing (R) conditions. No band was observed in non-transfected (NT) cells. AU753 did not recognize the S protein by western blot.