# *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.

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

| ABCD | Clone | Epitope | Reference | Yield (mg/L) |
|---|---|---|---|---|
| AS739 | S309 | S1/RBD | Pinto *et al*., 2020 | 100 |
| AT693 | BD-23 | | Cao *et al*., 2020 | 80 |
| AU197 | 2B04 | | Alsoussi *et al*., 2020 | 30 |
| AU734 | 2-43 | | Liu *et al*., 2020 | 40 |
| AU753 | MAb362 | | Ejemel *et al*., 2020 | 10 |

**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% (v/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.

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## Conflict of interest

The authors declare no conflict of interest.

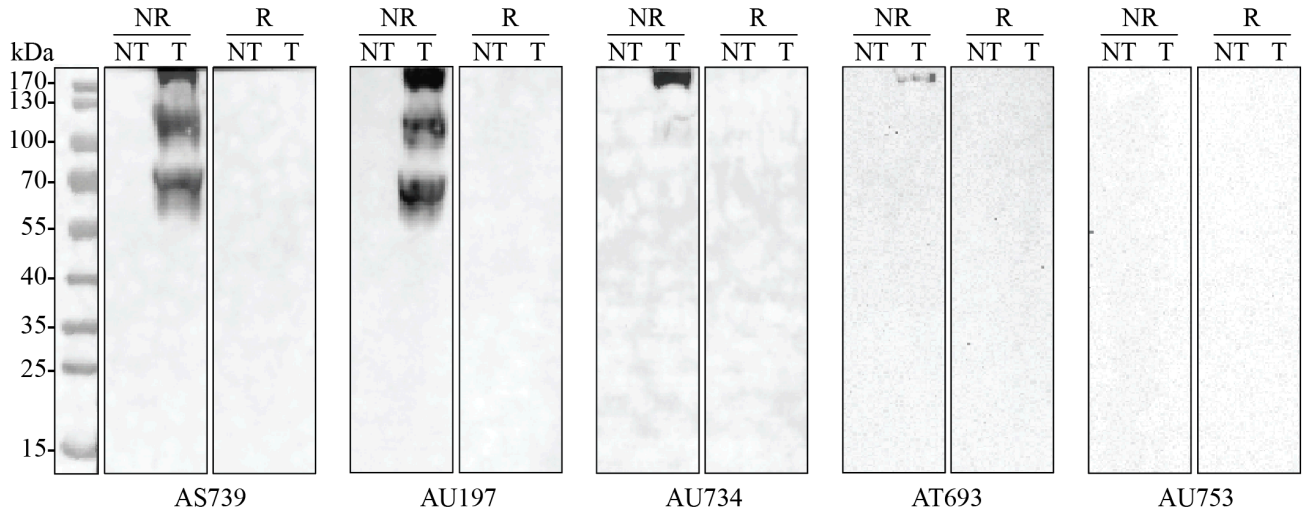


**Fig. 1.** Specific binding of AS739, AU197, AU734 and AT693 antibodies to the SARS-CoV-2 S protein in Vero-B4 transfected (T) cells in non-reducing (NR) conditions, but not in reducing (R) conditions. No band was observed in non-transfected (NT) cells. AU753 did not recognize the S protein by western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