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

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Abstract
The recombinant antibodies AS739, AT693 and AU734 detect by immunofluorescence 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 immunofluorescence. Three antibodies (AS739, AT693 and AU734) detected specifically the S protein from SARS-CoV-2; two others (AU197 and 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 3 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). Non-transfected cells were used as a negative control.

Protocol: Transfected Vero-B4 cells were fixed with methanol at -20 °C for 2 min. Fixed cells were washed once in PBS and once in PBS + 0.2% (w/v) BSA (PBS-BSA) during 10 min, and then incubated with the anti-S antibodies (final concentration 5 mg/L in PBS-BSA) for 30 min. After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes A11034). After 3 washes (10 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka 33480). Pictures were taken using a Zeiss LSM800 confocal microscope, with a 63x Neofluar oil immersion objective.

Results
Antibodies AS739, AT693 and AU734 specifically detected a signal in Vero-B4 cells expressing the SARS-CoV-2 S protein (Fig. 1). The absence of staining in non-transfected cells indicated the specificity of the signal observed. The distribution observed is consistent with a presence mostly in the early secretory pathway (endoplasmic reticulum and Golgi apparatus). AU753 did not recognize the S protein; this is possibly due to the fact that this antibody is poorly produced. A non-specific signal was observed with the AU197 antibody in non-transfected cells.

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Conflict of interest

The authors declare no conflict of interest.

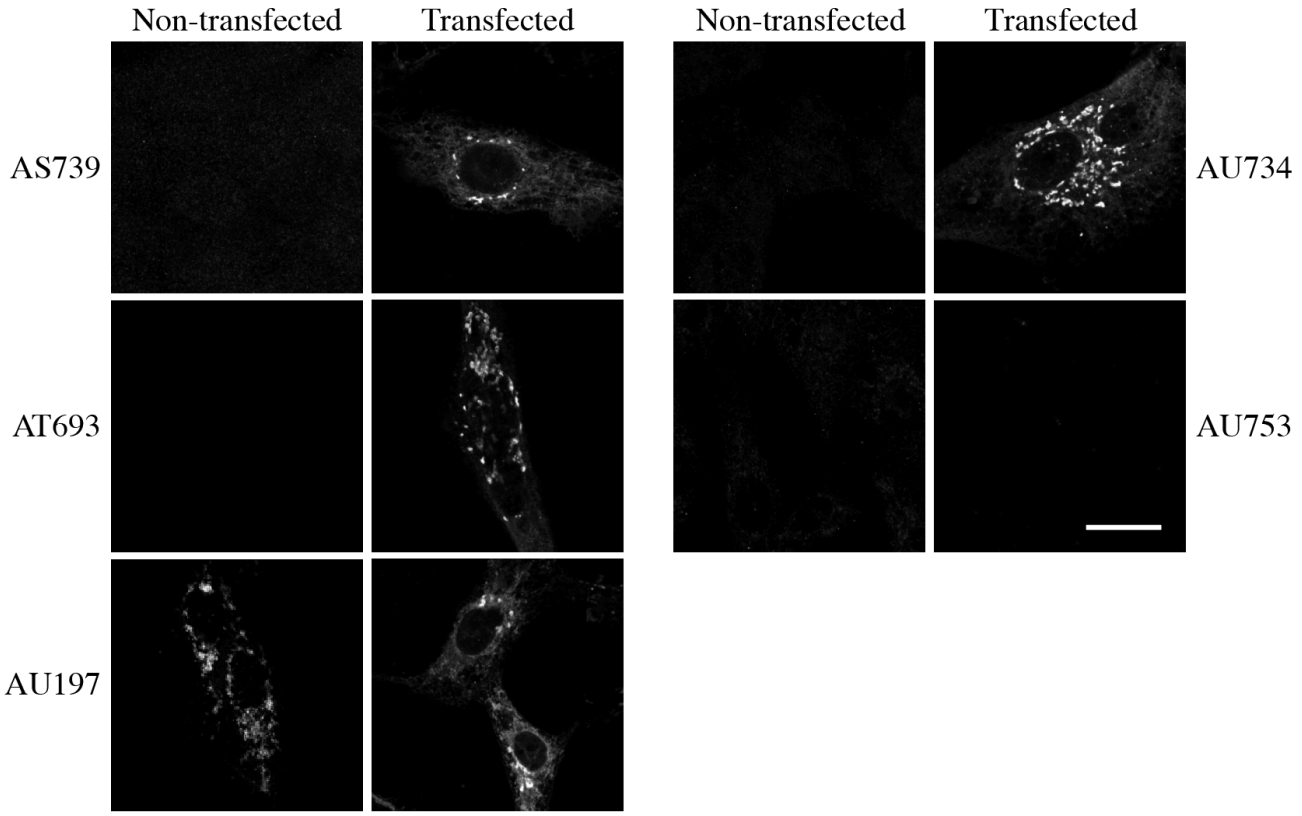

Fig. 1. AS739, AT693 and AU734 specifically labeled transfected Vero-B4 cells expressing the SARS-CoV-2 S protein. AU753 did not recognize the S protein. No labeling was seen in non-transfected cells except with the antibody AU197, which gives a non-specific signal in these conditions. Scale bar: 20 μm.