AQ806, AS739, AT693, AU197 and AU734 antibodies recognize the spike S protein from SARS-CoV-2 by flow cytometry

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Abstract
The recombinant antibodies AQ806, AS739, AT693, AU197 and AU734 detect by flow cytometry 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). Six recombinant antibodies recognizing the S1 domain of the S protein from SARS-CoV-2 (AQ806, AS739, AT693, AU197, AU734 and AU753) were tested for their ability to recognize the S protein by flow cytometry. Five antibodies (AQ806, AS739, AT693, AU197 and AU734) detected the S protein from SARS-CoV-2; one (AU753) did not.

Materials & Methods
Antibodies: ABCD_AQ806, 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)
AQ806	VHH-72	S1/RBD	Wrapp *et al*., 2020	100
AS739	S309		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: 10^6 transfected cells were pelleted and fixed with 250 µL of fixation solution (BD Biosciences 554714). After 20 min of incubation on ice, cells were pelleted and washed twice with 1 mL of Perm/Wash buffer (BD Biosciences 554714). Cells were then incubated for 30 min with the recombinant antibodies diluted at 1 mg/L in Perm/Wash buffer. After two washes in Perm/Wash buffer, cells were incubated for 20 min with the secondary goat anti-rabbit IgG conjugated to Alexa Fluor 488 (1:400 in Perm/Wash buffer; Molecular Probes #A11034). After three washes in Perm/Wash buffer, cells were resuspended in 500 µL of Perm/Wash buffer and analyzed with a flow cytometer (BD AccuriTM C6).

Results
Antibodies AQ806, AS739, AT693, AU197 and AU734 detected the SARS-CoV-2 spike S protein in Vero-B4 transfected cells. No signal was detected in non-transfected cells (Fig. 1). AU753 did not recognize the S protein by flow cytometry; this is possibly due to the fact that this antibody is poorly produced.

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Conflict of interest

The authors declare no conflict of interest.

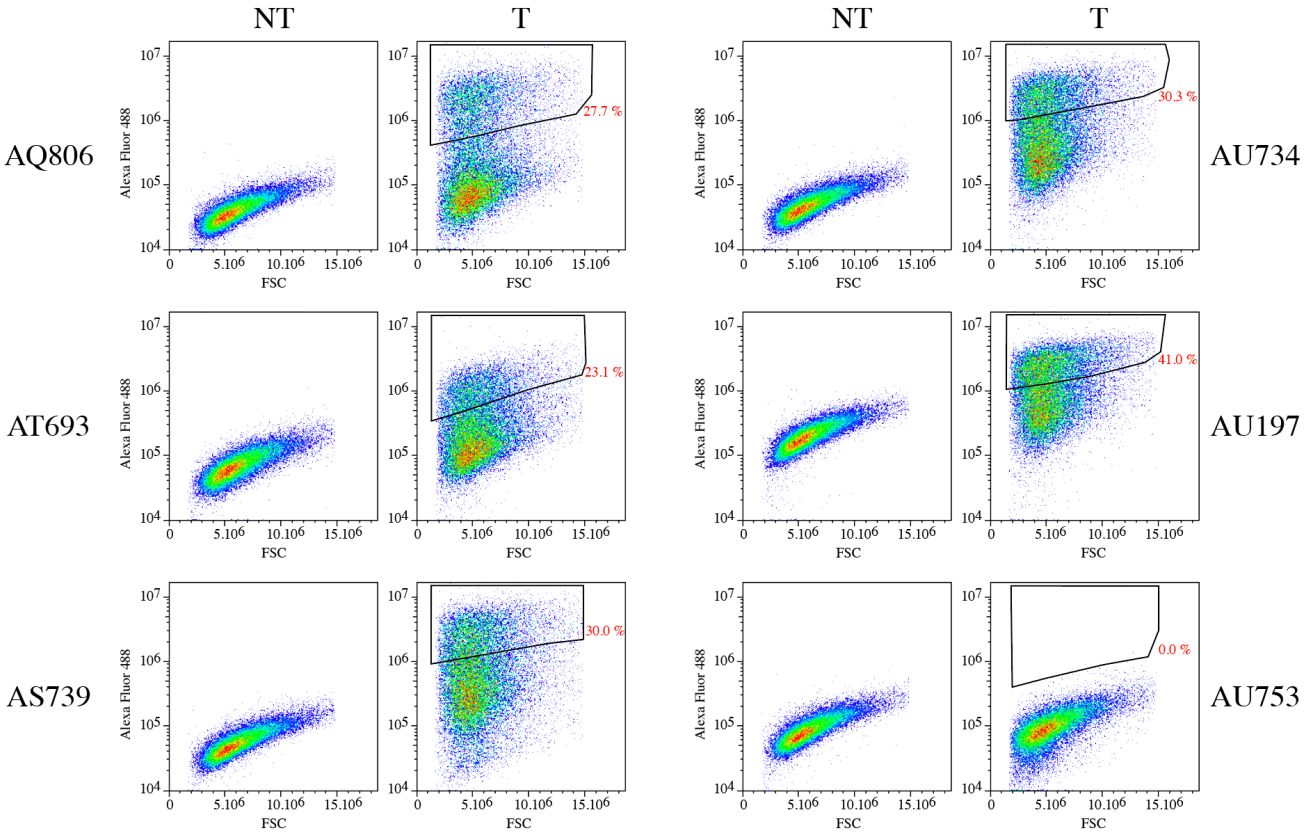

Fig. 1. Bi-parametric representation of flow cytometry analysis depicting Forward Scatter (FSC) and Alexa Fluor 488 signal. AQ806, AS739, AT693, AU197 and AU734 antibodies labeled Vero-B4 transfected cells (T) overexpressing the SARS-CoV-2 spike S protein. No signal was detected in non-transfected cells (NT). AU753 did not recognize the S protein by flow cytometry. Transfection efficiency was estimated to be around 30%.