AI334, AQ806 and RB596 antibodies recognize the spike S protein from SARS-CoV-2 by immunofluorescence

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Abstract
The recombinant antibodies AI334, AQ806 and RB596 detect by immunofluorescence 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). Three recombinant antibodies (AI334, AQ806 and RB596) successfully detect by immunofluorescence the S protein from SARS-CoV-2 (UniProt P0DTC2) expressed in Vero-B4 cells.

Materials & Methods
Antibodies: ABCD_AI334, ABCD_AQ806 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 scFv portion fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) of antibodies AI334 and AQ806 correspond to the sequences of the variable regions of the clones CR3022 (ter Meulen *et al.*, 2006) and VHH-72 (Wrapp *et al.*, 2020), respectively. Antibodies RB596 was raised via phage display against the SARS-CoV-2 S protein (Hammel *et al.*, 2020). HEK 293T 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 (30-100 mg/L) were collected after 5 days.

Antigen: Vero-B4 adherent cells (growing in DMEM, Gibco #11960044, supplemented with 10% FBS), were transiently transfected 24 h 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). Transfected cells were then seeded on multi-test glass slides (Thermo Fisher #15546375), and used to detect the viral protein. Non-transfected cells were used as a negative control.

Protocol: Transfected Vero-B4 cells were fixed with ice-cold Acetone/Methanol (ratio 1:1) for 10 min, and slides rehydrated for 10 min in PBS + 0.1% Tween20 (w/v) (PBS-T). Cells were then blocked in PBS-T + 0.2% BSA (w/v) for 30 min, and then incubated with the anti-S antibodies (final concentration 5 mg/L in PBS-T + BSA) for 1 h. After 3 washes with PBS-T, cells were incubated for 30 min in PBS-T + BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11029). After 3 washes with PBS-T, slides were briefly rinsed with dH_2O, and mounted with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results
AI334, AQ806 and RB596 antibodies specifically detected a signal in Vero-B4 cells transfected with the SARS-CoV-2 S protein (Fig. 1). The distribution observed is consistent with a presence mostly in the early secretory pathway (endoplasmic reticulum and Golgi apparatus). The specificity of the signal was verified by the absence of staining in non-transfected cells (Fig. 1).

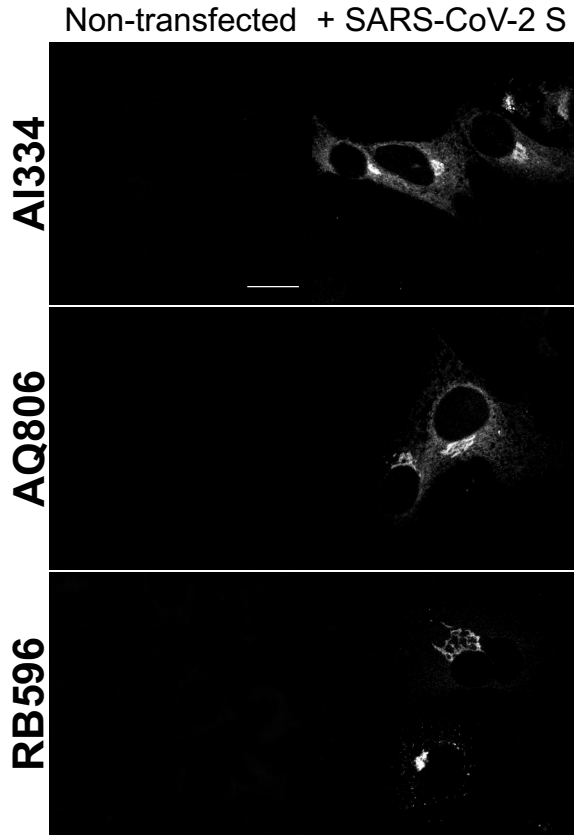

Fig. 1. Antibodies AI334, AQ806 and RB596 specifically labeled Vero-B4 cells expressing the SARS-CoV-2 S protein. No labeling was seen in non-transfected cells. Scale bar: 20 µm.

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Conflict of interest

The authors declare no conflict of interest.

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