AU304 and RB576 antibodies recognize the membrane M protein from SARS-CoV-1 and -2 by immunofluorescence

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Abstract
The recombinant antibodies AU304 and RB576 detect by immunofluorescence the membrane M protein from SARS-CoV-1 and SARS-CoV-2.

Introduction
The SARS-CoV membrane (M) protein is an abundant structural protein, driving virus assembly and budding into the lumen of the endoplasmic reticulum-Golgi intermediary compartment (ERGIC) and interacting with the E, S and N proteins (Siu *et al.*, 2008). Here we describe the ability of two recombinant antibodies (AU304 and RB576) to successfully detect by immunofluorescence the M protein from SARS-CoV-1 (UniProt P59596) and SARS-CoV-2 (UniProt P0DTC5) expressed in Vero-B4 cells.

Materials & Methods
Antibodies: ABCD_AU304 and ABCD_RB576 antibodies (https://web.expasy.org/abcd/, ABCD nomenclature) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a mouse IgG2A Fc. The synthesized scFv sequence (GeneArt, Invitrogen) of AU304 corresponds to the sequence of the variable regions of the clone SARSFab20 (Liang *et al.*, 2005). RB576 was raised against a synthetic peptide from the SARS-CoV-2 M protein (Hammel and Zenhausern, 2020). HEK 293T suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for each antibody. Supernatants (50-100 mg/L) were collected after 4 days.

Antigen: Vero-B4 cells (growing in DMEM [Gibco 31966], supplemented with 10% FBS), cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and transiently transfected 2 days before the experiment with an expression vector coding for the full-length SARS-CoV-1 or SARS-CoV-2 M protein, were used to detect the viral protein. Cells transfected with a vector coding for full-length SARS-CoV-2 E protein (UniProt P0DTC4) were used as a negative control.

Protocol: Transfected Vero-B4 cells were fixed with methanol at -20 °C for 3 min. Fixed cells were washed once in PBS and once with PBS + 0.2% (w/v) BSA (PBS-BSA) during 5 min, and then incubated with the anti-M antibodies (final concentration 5 mg/L in PBS-BSA) for 1 h. After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes A11029). 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 LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results
AU304 and RB576 antibodies specifically detected a signal in Vero-B4 cells expressing either the SARS-CoV-1 or the SARS-CoV-2 M protein, but not in cells expressing the control E protein (Fig. 1). The absence of staining in non-transfected cells indicated the specificity of the signal observed (Fig. 1, arrowheads). It should be stressed that in these experiments the signal observed was weak, indicating either that the affinity of the antibodies for the proteins was weak, or that the amount of protein present in transfected cells was low.

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Conflict of interest
The authors declare no conflict of interest.

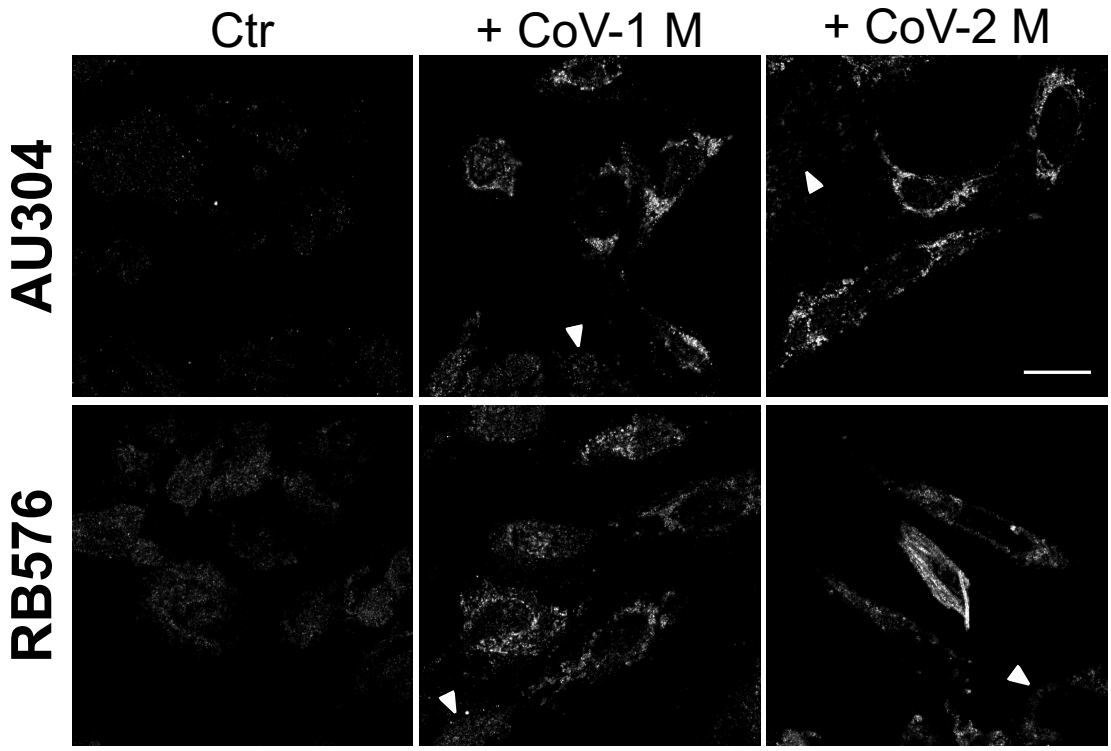

Fig. 1. Antibodies AU304 and RB576 specifically labeled Vero-B4 cells expressing the SARS-CoV-1 M protein ('+CoV-1 M') or the SARS-CoV-2 M protein ('+CoV-2 M'), but not cells expressing as a control the irrelevant E protein ('Ctr'). Arrowheads indicate unlabeled non-transfected cells. Scale bar: 20 μm.