RB572, RB574 and RB576 antibodies recognize the membrane M protein from SARS-CoV-2 by immunofluorescence

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Abstract
The recombinant antibodies RB572, RB574 and RB576 detect by immunofluorescence the membrane M protein from SARS-CoV-2.

Introduction
The SARS-CoV membrane (M) protein is the most 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 three recombinant antibodies (RB572, RB574 and RB576) to successfully detect by immunofluorescence the M protein from SARS-CoV-2 (UniProt P0DTC5) expressed in Vero-B4 cells.

Materials & Methods
Antibodies: ABCD_RB572, ABCD_RB574 and ABCD_RB576 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 VHH portion fused to a mouse IgG2A Fc (Hammel and Zenhausern, 2020). HEK 293T suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the VHH-Fc of 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-2 M protein, were used to detect the viral protein. Cells transfected with a vector coding for full-length SARS-CoV-2 E protein were used as a negative control.

Protocol: Two different fixation steps were used. Transfected Vero-B4 cells were either **(i)** fixed in PBS + 4% paraformaldehyde (w/v) (Applichem, A3013) for 30 min, blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, A3661) for 5 min, and then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, S7900) for 3 min; or **(ii)** 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
RB572, RB574 and RB576 antibodies specifically detected a signal in Vero-B4 cells expressing the SARS-CoV-2 M protein, but not in cells expressing the control E protein (Fig. 1). The specificity of the signal could be verified by the absence of staining in non-transfected cells (Fig. 1, arrowheads). A specific signal was detected only when cells were fixed with methanol (Fig. 1, MeOH column), but not when paraformaldehyde was used (Fig. 1, PAF column).

References
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Conflict of interest
The authors declare no conflict of interest.

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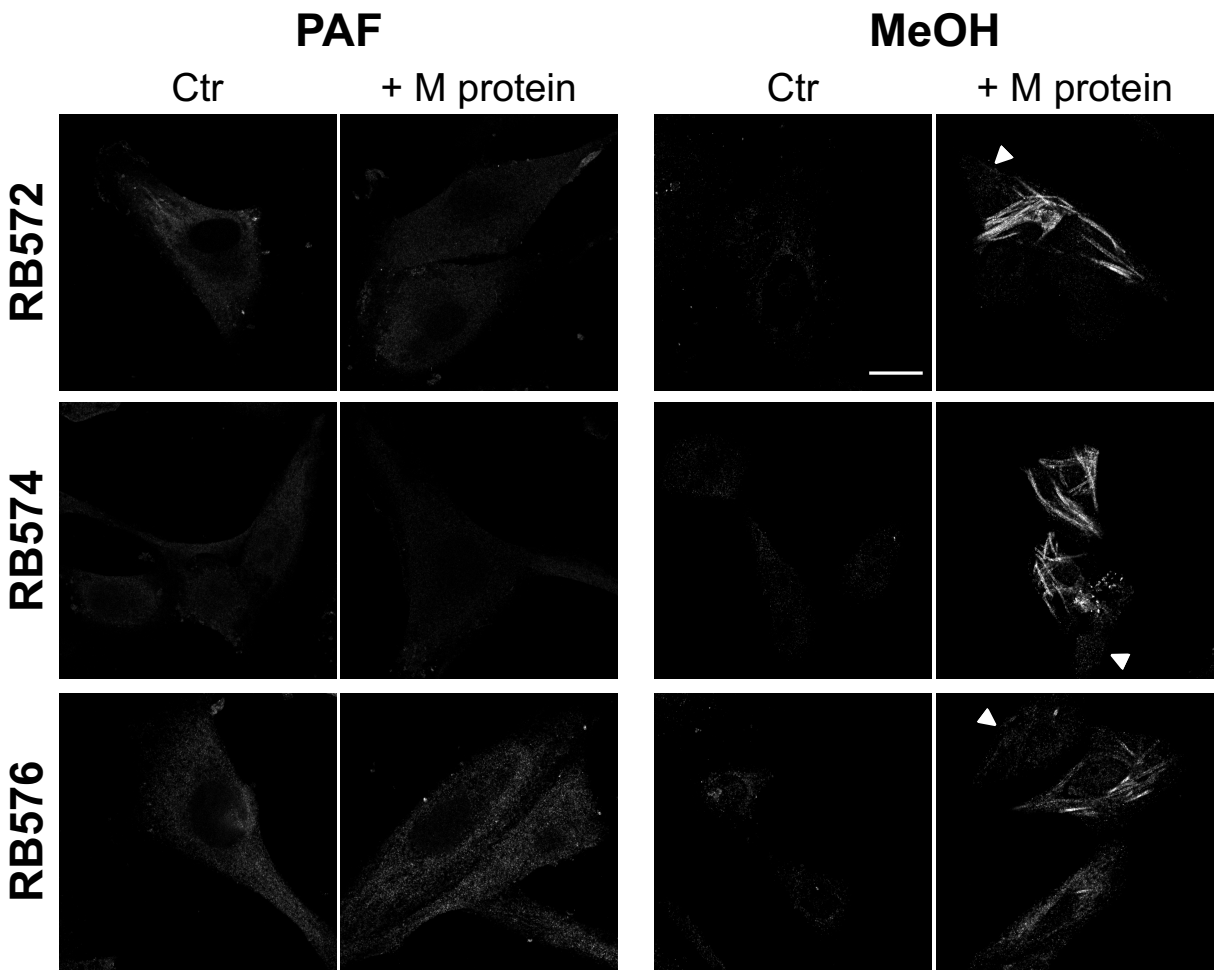

Fig. 1. Antibodies RB572, RB574 and RB576 specifically labeled Vero-B4 cells expressing the SARS-CoV-2 M protein ('+M protein'), but not cells expressing the control E protein ('Ctr') in methanol-fixed cells (MeOH column). Arrowheads indicate unlabeled non-transfected cells. The same antibodies do not show a specific signal in cells expressing the M protein ('+M protein') compared to control cells ('Ctr') in paraformaldehyde-fixed cells (PAF column). Scale bar: 20 µm.

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