The RB621 antibody recognizes the envelope E protein from SARS-CoV-2 by immunofluorescence

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Abstract

The recombinant antibody RB621 detects by immunofluorescence the envelope E protein from SARS-CoV-2.

Introduction

The SARS-CoV envelope (E) protein is a minor structural protein, implicated in viral morphogenesis, assembly and budding. It is a short, integral membrane protein, potentially able to adopt multiple membrane topologies and orientations (Schoeman and Fielding, 2019). Here we describe the ability of the recombinant antibody RB621 to successfully detect by immunofluorescence the E protein from SARS-CoV-2 (UniProt P0DTC4) expressed in Vero-B4 cells.

Materials & Methods

Antibodies: ABCD_RB621 antibody (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as a mini-antibody 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. Supernatant (~100 mg/L) was 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 E protein, were used to detect the viral protein. Cells transfected with a vector coding for full-length SARS-CoV-2 M protein were used as a negative control.

Protocol: Two different fixation procedures 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, washed once in PBS and once with PBS + 0.2% (w/v) BSA (PBS-BSA) during 5 min. Fixed cells were then incubated with RB621 (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

The RB621 antibody specifically detected a signal in Vero-B4 cells expressing the SARS-CoV-2 E protein, but not in cells expressing the control M 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), not when paraformaldehyde was used (Fig. 1, PAF).

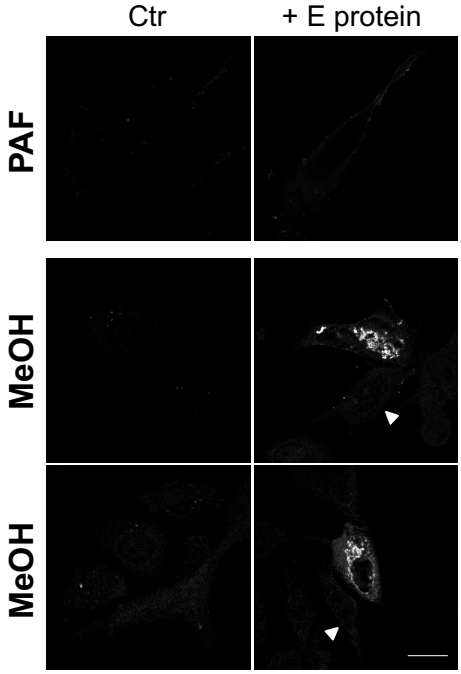

Fig. 1. RB621 specifically labeled Vero-B4 cells expressing the SARS-CoV-2 E protein ('+E protein'), but not cells expressing the control M protein ('Ctr') in methanol-fixed cells ('MeOH'). Arrowheads indicate unlabeled non-transfected cells. Scale bar: 20 µm.

References

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