The RB596 antibody recognizes the spike S protein from SARS-CoV-2 by ELISA

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Abstract
The recombinant antibody RB596 detects by ELISA the spike S protein from SARS-CoV-2.

Introduction
The spike (S) glycoprotein mediates 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). Here we describe the ability of the recombinant antibody RB596 to detect by ELISA the soluble ectodomain of the S protein from SARS-CoV-2 (UniProt P0DTC2).

Materials & Methods
Antibodies: ABCD_RB596 antibody (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding VHH portion fused to a mouse IgG2A Fc. HEK293 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 5 days.

Antigen: The antibody was raised against the ectodomain (residues 1-1208) of the SARS-CoV-2 S protein, with a KV->PP substitution at residues 986/987, a RRAR->GSAS substitution at residues 682-685, and C-terminal T4 fibritin trimerization motif, protease cleavage site, TwinStrepTag and 8xHisTag (PDB #6VSB; Wrapp et al., 2020). As a negative control, an irrelevant protein (GOLPH3, Golgi phosphoprotein 3, UniProt Q9H4A6), also containing the TwinStrep and 8xHis tags, was used.

Protocol: Antigen proteins (10 µg/ml, 50 µl/well in PBS 0.5% (w/v) BSA, 0.1% (w/v) Tween20) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of RB596 supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Bio-Rad #170-6516, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H2SO4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results
The RB596 antibody bound in a concentration-dependent manner to the SARS-CoV-2 S protein, but not to an unrelated tagged protein (Fig. 1). This antibody has also been shown to recognize the full-length SARS-CoV-2 S protein by immunofluorescence (Marchetti et al., 2020).

Fig. 1. Specific binding of RB596 to the SARS-CoV-2 S protein, but not to the negative control protein, as detected by ELISA.

References

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Conflict of interest
The authors declare no conflict of interest.