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 H_2SO_4. 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).

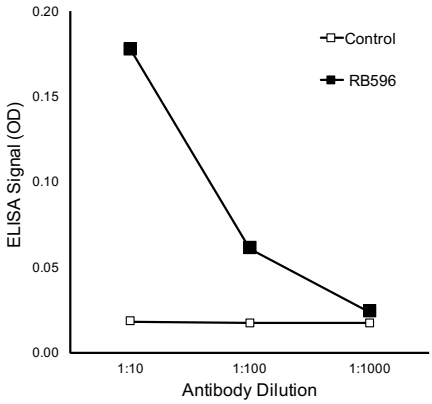

Fig. 1. Specific binding of RB596 to the SARS-CoV-2 S protein, but not to the negative control protein, as detected by ELISA.

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Conflict of interest
The authors declare no conflict of interest.

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