RB519, RB520, RB521 and RB522 antibodies recognize the human ISOC1 protein by ELISA

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Abstract

The recombinant antibodies RB519, RB520, RB521 and RB522 detect by ELISA the human ISOC1 fused to a GST protein.

Introduction

ISOC1 (Isochorismatase domain-containing protein 1, UniProtKB #Q96CN7) is a protein expressed in the peroxisome of different subsets of human cells (Gronemeyet *et al.*, 2013; Islinger *et al.*, 2007). Here we describe the ability of four recombinant antibodies (RB519, RB520, RB521 and RB522) to detect by ELISA a GST-fused ISOC1 protein.

Materials & Methods

Antibodies: ABCD_RB519, ABCD_RB520, ABCD_RB5521 and ABCD_RB522 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc (RRB519, RRB520, RRB521 AND RRB522). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~20-100 mg/l) were collected after 4 days.

Antigen: The antibodies were originally raised against a GST protein fused to the full-length ISOC1 protein (residues 2-298). This chimeric GST-ISOC1 was used as antigen for ELISA detection. GST was used as negative control.

Protocol: The whole procedure was carried out at room temperature. Bacterial lysates containing GST proteins were incubated in a glutathione-coated 96-well plate (Pierce #15240) 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 RRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit 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

Antibodies RRB519, RRB520, RRB521 and RRB522 bound in a concentration-dependent manner to the GST-ISOC1 antigen, but not to the GST negative control (Fig. 1).

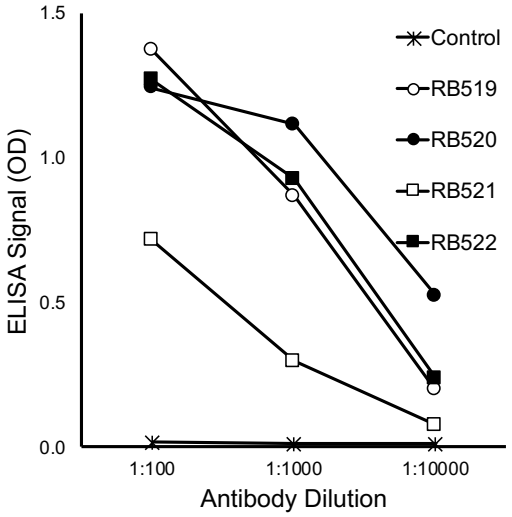

Fig. 1. Specific binding of RRB antibodies to the target GST-ISOC1 protein, as detected by ELISA. 'Control' indicates the binding of RRB519 to GST (all other control curves were superimposed).

References

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Conflict of interest

The authors declare no conflict of interest.