RB501, RB502, RB503, RB504 and RB505 antibodies recognize the human UNC93B1 protein by ELISA

Jennifer Wen-An Wang, Nicolas Demaurex

Dept. of Physiology and Metabolism, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies RB501, RB502, RB503, RB504 and RB505 detect by ELISA the human Protein unc-93 homolog B1fused to a GST protein.

Introduction

The protein uncoordinated 93 homolog B1 (UNC93B1, Uniprot #Q9H1C4) is a human twelve-transmembrane protein localized in the endoplasmic reticulum membrane and endosomal compartments (Maschalidi *et al.*, 2017). Here we describe the ability of five recombinant antibodies (RB501, RB502, RB503, RB504 and RB505) to detect by ELISA a GST-fused UNC93B1 protein.

Materials & Methods

Antibodies: ABCD_RB501, ABCD_RB502, ABCD_RB503, ABCD_RB504 and ABCD_RB505 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 (RRB501, RRB502, RRB503, RRB504, RRB505). 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 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the residues 2-63 of UNC93B1. This chimeric *in vivo* biotinylated GST-UNC93B1 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 streptavidin-coated ELISA plates (Pierce #15124) for 30 min at 4°C. 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 RRB501, RRB502, RRB503, RRB504 and RRB505 bound in a concentration-dependent manner to the GST-UNC93B1 antigen, but not to the GST negative control (Fig. 1).

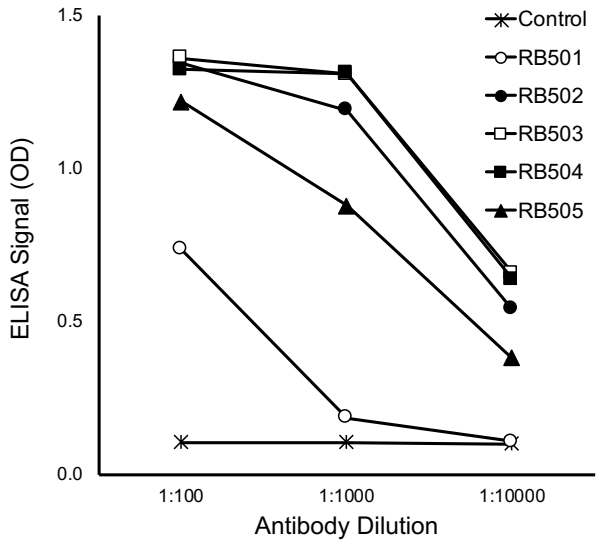

Fig. 1. Specific binding of RRB antibodies to the target GST-UNC93B1 protein, as detected by ELISA. 'Control' indicates the binding of RRB501 to GST (all other control curves were superimposed).

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014; 31(1):37-42. PMID:24100547

Maschalidi S, Nunes-Hasler P, Nascimento CR, *et al*. UNC93B1 interacts with the calcium sensor STIM1 for efficient antigen cross-presentation in dendritic cells. Nat Commun. 2017; 8(1):1640. PMID:29158474

Conflict of interest

The authors declare no conflict of interest.