RB815, RB816 and RB817antibodies recognize the Ripply2 protein from Danio rerio by ELISA

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Abstract

The recombinant antibodies RB815, RB816 and RB817 detect by ELISA the *Danio rerio* Ripply2 protein fused to a Twin-Strep-Tag.

Introduction

Ripply2 (UniProt #Q2WG79) is a *Danio rerio* protein involved in somitogenesis (Kawamura *et al*., 2005). Here we describe the ability of three recombinant antibodies (RB815, RB816 and RB817) to detect by ELISA the Ripply2 protein.

Materials & Methods

Antibodies: ABCD_RB815, ABCD_RB816 and ABCD_RB817 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/) and produced as mini-antibodies with the antigen-binding scFv portion fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) comprise the sequence of the variable regions joined by the linker (GGGS)3. HEK293 suspension cells growing in HEK TF medium (Xell#861-0001, Sartorius) supplemented with 0.1% Pluronic F68 (Sigma #P1300) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~70 mg/L, 80 mg/L and 40 mg/L for RB815, RB816 and RB817, respectively) were collected after 5 days.

Antigen: Antibodies were raised against the full-length Ripply2 protein, fused at its C-terminus with a Twin-Strep-Tag (RIPP2-TST) and produced in transiently transfected HEK293 cells. The same construction was used for the ELISA experiment. A Twin-Strep-Tagged peptide from the human tenascin protein served as a negative control (UniProt #P24821, residues 1619-1710, TNC-TST).

Protocol: The whole procedure was carried out at room temperature. TST tagged proteins at saturating concentration (10 pmol/well) were incubated on MaxiSorp 96-well plates (Nunc # 44-2404-21) 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 RB 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 (Sigma #A8275, 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 RB815, RB816 and RB917 bound in a concentration-dependent manner to the RIPP2-TST protein against which they were raised, but not to the negative control protein (Fig. 1).

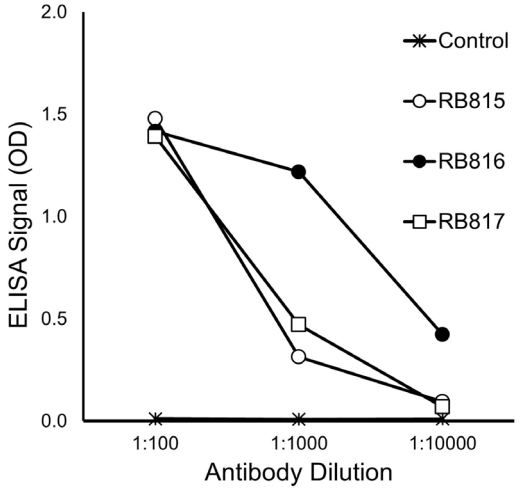

Fig. 1. Specific binding of RB antibodies to the target RIPP2-TST protein, as detected by ELISA. 'Control' indicates the binding of RB815 to the negative control peptide TNC-TST (all other control curves were superimposed).

References

Kawamura A, Koshida S, Hijikata H, Ohbayashi A, Kondoh H, Takada S. Groucho-associated transcriptional repressor ripply1 is required for proper transition from the presomitic mesoderm to somites. Dev Cell. 2005 Dec;9(6):735-44. PMID: 16326386.

Conflict of interest

Philippe Hammel is a cofounder and a shareholder of ABCD Antibodies SA.