

RB740, RB741 and RB742 antibodies recognize the *Dictyostelium discoideum* Talin-B protein by ELISA

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Abstract

The recombinant antibodies RB740, RB741 and RB742 detect by ELISA the *Dictyostelium discoideum* Talin-B fused to a GST protein.

Introduction

Talin-B (TalB, UniProt #Q54K81) is a *D. discoideum* actin-binding protein which is involved in phagocytosis (Niewöhner *et al.*, 1997) and morphogenesis (Tsujioka *et al.*, 2004). Here we describe the ability of three recombinant antibodies (RB740, RB741 and RB742) to detect by ELISA a GST-fused TalB protein.

Materials & Methods

Antibodies: ABCD_RB740, ABCD_RB741 and ABCD_RB742 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) and produced as mini-antibodies with the antigen-binding scFv used to a mouse IgG2A Fc (MRB740, MRB741 and MRB742). HEK293 suspension cells (growing in HEK TF medium, Xell#861-0001, supplemented with 0.1% Pluronic F68, Sigma#P1300) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~100 mg/L) were collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the residues 737 to 876 of TalB protein. This chimeric GST-TalB 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 μ L of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 μ L of MRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 μ L washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Bio-Rad #170-6516, dilution 1:1000, 50 μ L per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 μ L per well). The

reaction was stopped by the addition of 25 μ L of 2 M H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies MRB740, MRB741 and MRB742 bound in a concentration-dependent manner to the GST-TalB antigen, but not to the GST negative control (Fig. 1).

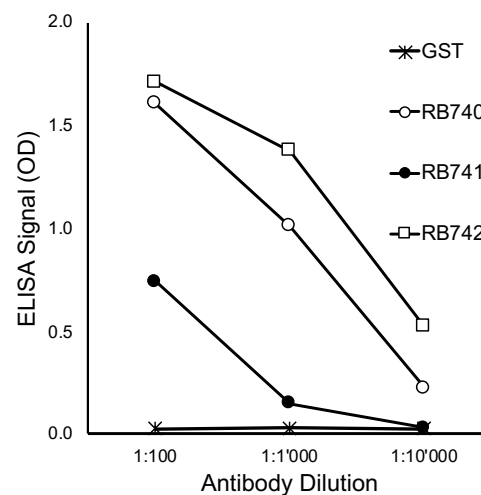


Fig. 1. Specific binding of MRB antibodies to the target GST-TalB protein, but not to GST (shown only for MRB740; MRB741 and MRB742 background curve is superimposed), as detected by ELISA.

References

- Niewöhner J, Weber I, Maniak M, Müller-Taubenberger A, Gerisch G. Talin-null cells of *Dictyostelium* are strongly defective in adhesion to particle and substrate surfaces and slightly impaired in cytokinesis. *J Cell Biol.* 1997 Jul 28;138(2):349-61. PMID: 9230077.
- Tsujioka M, Yoshida K, Inouye K. Talin B is required for force transmission in morphogenesis of *Dictyostelium*. *EMBO J.* 2004 Jun 2;23(11):2216-25. PMID: 15141168

Conflict of interest

The authors declare no conflict of interest.