

The RB030 antibody recognizes a peptide of the *D. discoideum* TspB protein by western blot

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Abstract

The recombinant antibody RB030 detects by western blot a peptide of the *Dictyostelium discoideum* TspB protein fused to a GST protein; RB029 does not.

Introduction

Tetraspanins, characterized by four transmembrane domains and a large extracellular loop (EC2) containing a conserved CCG-motif, potentially function as regulators of cellular signaling (Albers *et al.*, 2016). Here we describe the ability of the RB030 antibody to detect by western blot a fragment of the TspB protein (DDB_G0269872, UniProt #Q55CW7), one of the five members of the *Dictyostelium* tetraspanin family, fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RB029 and ABCD_RB030 antibodies (ABCD nomenclature, web.expasy.org/abcd; Lima *et al.*, 2020) 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 mouse IgG2A Fc (MRB029 and MRB030). HeLa cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~1 mg/L) were collected after 4 days.

Antigen: The antibodies were originally raised against a GST protein fused to 47 residues (ENFLKCCYWNSTSSRNPL LCPKDSKGIPKYTDTCDSVISSKISSN) of the TspB EC2 domain. This chimeric GST-TspB protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-TspB recombinant protein was induced in *E. coli* bacteria growing exponentially (OD₆₀₀, 0.5) at 37°C (in 50 ml of Luria-Bertani (LB) medium containing 20% glucose and 100 μM ampicillin) by addition of 1.5 mM IPTG. After 3 h, bacteria were pelleted and resuspended in lysis buffer (4 ml of PBS + 1% Triton X100 + aprotinin 10 μg/ml + leupeptin 20 μg/ml + iodoacetamide 1.8 mg/ml + PMSF 18 μg/ml) and lysed by sonication. GST was purified on glutathione-coupled sepharose 4 Fast Flow beads (GE Healthcare Life Sciences #17-5132-01), then eluted in 500 μl of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 15 μL of each sample was migrated (200 V, 30 min) in a 12% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1043), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes

(iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked overnight at 4 °C in PBS containing 0.1% (v/v) Tween20 and 5% (w/v) milk, and washed three times (5 minutes) in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (undiluted), for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse (Biorad #170-6516, dilution 1:3000) for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) using a PXi-4 gel imaging systems (Syngene).

Results

The RB030 antibody specifically recognizes the GST-TspB fusion protein (~30 kDa), as well as a probable partial degradation product at ~18 kDa; it does not bind the GST negative control (Fig. 1). RB029 does not recognize GST-TspB (Fig. 1).

Note that the antigen used here encompasses a fragment of an extracellular domain of the TspB protein, and it is presumably not properly folded. It may also lack proper post-translational modifications. Further experiments will be necessary to determine if and in which experimental conditions RB030 recognizes the full-length TspB protein.

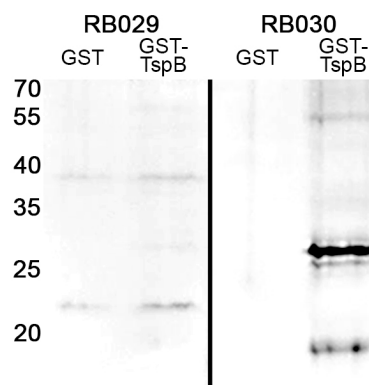


Fig. 1. Specific binding of the RB030 antibody to the GST-TspB protein (predicted molecular mass ~30 kDa).

References

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