

RA043 and RA044 antibodies recognize a peptide of the *D. discoideum* SibC protein by western blot

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Abstract

Recombinant antibodies RA043 and RA044 detect by western blot a peptide of the *Dictyostelium discoideum* SibC protein fused to a GST protein.

Introduction

SibC (Similar to iIntegrin Beta C, DDB_G0288195, UniProt #Q54JA5) is a protein similar to metazoan integrin beta chains, involved in cell adhesion in *D. discoideum* (Cornillon *et al.*, 2008). Here we describe the ability of the RA043 and RA044 antibodies to detect by western blot a fragment of the SibC protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RA043 and ABCD_RA044 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 (MRA043 and MRA044). 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 the last 47 cytosolic residues (RKAAPPTDTFFSEAAFLGDGVSSNPLYEQSASAAENPLYQSA SDTTD) of the SibC protein. This chimeric GST-SibC protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-SibC 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

RA043 and RA044 antibodies specifically recognize the GST-SibC fusion protein (~31 kDa). The antibodies do not bind the GST negative control (Fig. 1).

The antigen used is a short cytosolic domain. It presumably does not fold into a complex structure, nor contains post-translational modifications. Accordingly, it is likely that the antibodies will also recognize the full-length protein. Further experiments will be necessary to determine if this is the case, and in which experimental procedures these antibodies can be used.

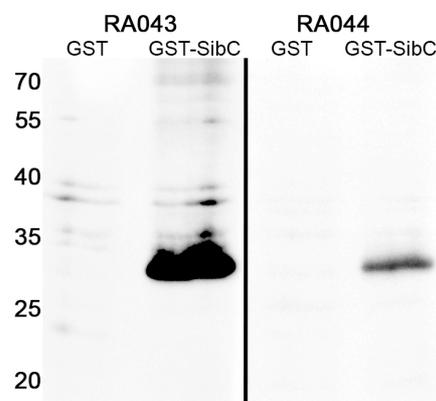


Fig. 1. Specific binding of RA043 and RA044 antibodies to the GST-SibC protein (predicted molecular mass ~31 kDa).

References

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