RB039, RB040 and RB042 antibodies recognize a peptide of the D. discoideum NoxB protein by western blot

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Abstract

Recombinant antibodies RB039, RB040 and RB042 detect by western blot a peptide of the *Dictyostelium discoideum* NoxB protein fused to a GST protein.

Introduction

NoxB (Superoxide-generating NADPH oxidase heavy chain subunit B, DDB_G0287101, UniProt #Q86GL4) is a subunit of the $gp91^{phox}$ complex in *D. discoideum* (Lardy *et al*., 2005). Here we describe the ability of the RB039, RB040 and RB042 antibodies to detect by western blot a fragment of the NoxB protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RB039, ABCD_RB040 and ABCD_RB042 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 (MRB039, MRB040 and MRB041). 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 21 residues (KNCNKFNGKNNCHLIFHKENF) of the NoxB protein. This chimeric GST-NoxB protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-NoxB recombinant protein was induced in *E. coli* bacteria growing exponentially (OD_{600}, 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

RB039 and RB042 antibodies, and to a lesser extent RB040, specifically recognize the GST-NoxB fusion protein (~29 kDa). The antibodies do not bind the GST negative control (Fig. 1).

Note that this antigen only encompasses a small portion of the NoxB protein, and that it is presumably not properly folded. Further experiments will be necessary to determine if and in what conditions these antibodies recognize the full NoxB protein.

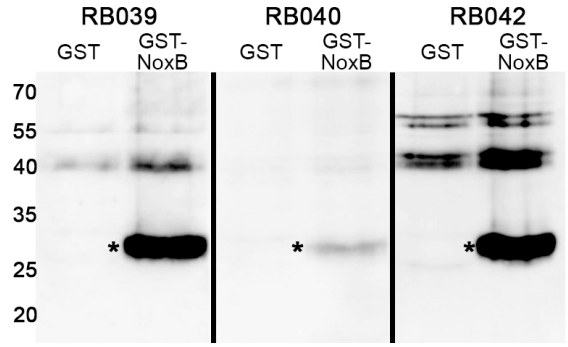

Fig. 1. Specific binding of RB039, RB040 and RB042 antibodies to the GST-NoxB protein (predicted molecular mass ~29 kDa).

References

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Conflict of interest

The authors declare no conflict of interest.