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The RA026 antibody recognizes a peptide of the D. discoideum NoxC protein by western blot

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Abstract

The recombinant antibody RA026 detects by western blot a peptide of the *Dictyostelium discoideum* NoxC protein fused to a GST protein; RA025 and RA027 do not.

Introduction

NoxC (Superoxide-generating NADPH oxidase heavy chain subunit C, DDB_G0291117, UniProt #Q54F44) is a subunit of the gp91^{phox} complex in *D. discoideum* (Lardy *et al*., 2005). Here we describe the ability of the RA026 antibody to detect by western blot a fragment of the NoxC protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RA025, ABCD_RA026 and ABCD_RA027 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 (MRA025, MRA026 and MRA027). HeLa cells (growing in DMEM GlutaMAX^{TM} (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 (`CRYYTCLKTGGTKFYFHKENF`) of the NoxC protein. This chimeric GST-NoxC protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-NoxC 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

The RA026 antibody specifically recognizes the GST-NoxC fusion protein (~29 kDa); it does not bind the GST negative control (Fig. 1). RA025 does not recognize GST-NoxC; RA027 shows only a very faint band of the expected size (Fig. 1).

Note that this antigen only encompasses a small portion of the NoxC protein, and that it is presumably not properly folded. Further experiments will be necessary to determine if and in what conditions the RA026 antibody recognizes the full NoxC protein.

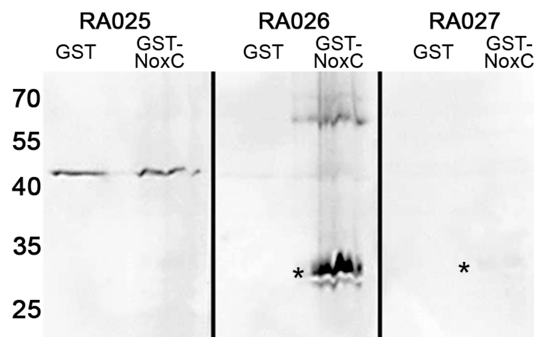

Fig. 1. Specific binding of the RA026 antibody to the GST-NoxC protein (predicted molecular mass ~29 kDa).

References

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Conflict of interest

The authors declare no conflict of interest.

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