The RB008 antibody does not recognize D. discoideum *NoxA by western blot*

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Abstract

The recombinant antibody RB008 does not detect by western blot the *Dictyostelium discoideum* protein NoxA expressed in *D. discoideum* and in two human cell-lines.

Introduction

The *D. discoideum* protein NoxA (UniProt Q9XYS3, DDB_G0289653) is a homologue of the catalytic subunit of the human NADPH oxidase complex (NOX2). In humans, NOX2 is responsible for the production of Reactive Oxygen Species (ROS), which can promote apoptosis and are important cell-autonomous defenses against intracellular pathogens (Dunn *et al.*, 2018). In humans, mutations subunits of the NOX2 complex cause chronic granulomatous disease (CGD), which is characterized by recurrent infections. Here, we describe that the recombinant antibody RB008 targeting a *D. discoideum* NoxA peptide was not able to detect the full-length protein expressed in *D. discoideum* and in two human cell lines by western blot.

Materials & Methods

Antibodies: ABCD_RB008 (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding scFv fused to a rabbit IgG Fc. HEK293 adherent cells (growing in DMEM, Gibco 11960044, supplied with 8% FBS) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (~1-5 mg/L) was collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein C-terminally fused to the residues 2-19 (RLPTKEEIQRYWVNEGNK) of the NoxA protein.

Protocol: *D. discoideum* and human cells (HEK293 and the PLB-985 [CGD] cell line differentiated in neutrophils [Pedruzzi *et al.,* 2002]) were collected, washed in Sorensen-120 mM Sorbitol, counted and resuspended as to have 1x10^7 cells/600µl in RIPA solution (20 mM Tris HCl pH 7.5, 150 mM NaCl, 0.1% SDS, 5 mM EDTA, 0.5% sodium deoxycholate, 1% triton X-100, 1x proteinase inhibitor cocktail [Sigma-Aldrich 11873580001]), mixed and incubated 30 min on ice. Cells were centrifuged and the supernatant was recovered. Protein content was measured by the Bradford assay. The samples were resuspended in 1x Laemmli Buffer (125 mM Tris pH 6.8, 4% (w/v) SDS, 20% glycerol, 0.01% (w/v) bromophenol blue, 10 mM DTT). 25 µg of each sample was migrated (50 V stacking and 150 V running, 1h30) in an 8% homemade acrylamide gel and transferred to a nitrocellulose membrane (Amersham, Protran GE10600002) in 25 mM Tris, 192 mM glycine, 20% MeOH, 0.01% SDS at 4 °C, 30 V, 16 h. After checking transfer by Ponceau Red staining, the membrane used for the recombinant antibody was blocked during 1 hour in PBS containing 5% (w/v) BSA (bovine serum albumin fraction V, pH 7.0 [SERVA Electrophoresis GmbH 11930]) and the second membrane to be probed with anti-GFP was blocked during 1 hour in PBS containing 5% (w/v) milk (GE Healthcare RPN418), 0.2% (w/v) Tween20. The first membrane was then incubated with RB008 (dilution 1:2 in PBS and 3% (w/v) BSA) and the second membrane with anti-GFP antibody (Abmart, M20004M, dilution 1:1000 in PBS with Tween and 3% (w/v) milk), overnight at 4 °C, then washed three times for 10 minutes in PBS with or without Tween. The membrane probed with the recombinant anti-NoxA antibody was then incubated during 1 h with goat anti-rabbit coupled to horseradish peroxidase (BioRad) in PBS and 3% (w/v) BSA) and anti-GFP with goat anti-mouse IgG coupled to horseradish peroxidase (Brunschwig, dilution 1:10'000 in PBS-Tween 3% (w/v) milk) and washed three times for 10 minutes in PBS with or without Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences RPN2232) using a Fusion Fx device (Vilbert Lourmat).

Results

The RB008 antibody directed against the *D. discoideum* NoxA was tested on lysates from *D. discoideum* Ax2-214 (Watts and Ashworth, 1970). ΔNoxABC cells, which lack all three Nox isoforms, were used as negative control (Zhang *et al.*, 2016), and Ax2-214 cells overexpressing the NoxA-GFP fusion protein as positive control. In addition, the antibody was tested on lysates from human cells, in which the native NoxA protein, codon-optimized for expression in human, was expressed using a lentiviral vector. The commercial anti-GFP recognized the tagged NoxA-GFP protein (Fig. 1) and also recognized nonspecifically a protein of approximately 80 kDa (asterisk), in *D. discoideum* lysates. The antibody RB008 recognized neither the GFP-tagged protein in *D. discoideum* cells nor the native untagged protein in human cells (Fig. 1). The RB008 antibody only recognized nonspecific background proteins.

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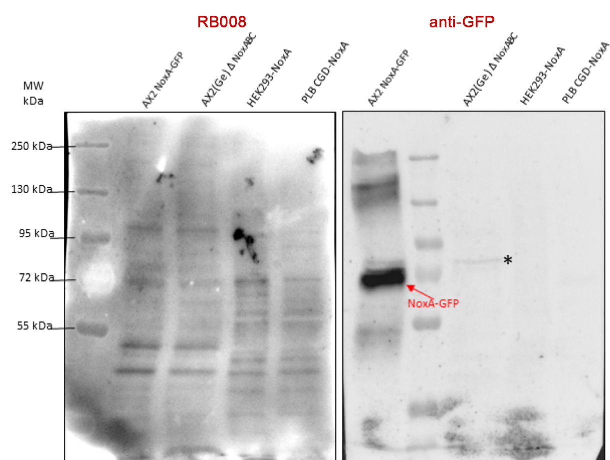

Fig. 1. Western blot on lysates from wild type *D. discoideum* Ax2-214 cells expressing NoxA-GFP, Ax2-214 ΔNoxABC cells (as negative control), and human HEK293 or PLB-985 (CGD) expressing untagged NoxA**.** Membranes were incubated with the indicated antibodies. The commercial anti-GFP antibody was used as a positive control. Endogenous NoxA has an expected size of ~60 kDa, and NoxA-GFP of ~85 kDa.

References

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Conflict of interest

The authors declare no conflict of interest.

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