RB285, RB286, RB287, RB288, RB289 and RB290 antibodies recognize a Dictyostelium *NcfA peptide by ELISA*

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Abstract

The recombinant antibodies RB285, RB286, RB287, RB288, RB289 and RB290 detect by ELISA a synthetic peptide from the *Dictyostelium* NcfA protein.

Introduction

NcfA (neutrophil cytosol factor A, or p67phox; DDB_G0288773, UniProt #Q867T7) is a NADPH oxidase activator of the amoeba *Dictyostelium discoideum*. Here we describe the ability of six recombinant antibodies (RB285, RB286, RB287, RB288, RB289 and RB290) to detect by ELISA a synthetic biotinylated peptide from the NcfA protein.

Materials & Methods

Antibodies: ABCD_RB285, ABCD_RB286, ABCD_RB287, ABCD_RB288, ABCD_RB289 and ABCD_RB290 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv portion fused to a rabbit Fc (RRB285, RRB286, RRB287, RRB288, RRB289 and RRB290). HEK293T 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 (~5 mg/L) were collected after 3 days.

Antigen: The N-biotinylated synthetic peptide, against which the antibodies were raised, corresponds to the 28 C-terminal residues of NcfA (YPYQVLYTDSNEKYYLNT ETNETFWELP). As a negative control, an irrelevant N-biotinylated peptide (GLLPVLESFKVSFLSALEEYTKK LNT) from human ApoA1 (UniProt #P02647) was used.

Protocol: The whole procedure was carried out at room temperature. Biotinylated peptides at saturating concentration (10 pmol/well) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of RRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma #A8275, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RRB285, RRB286, RRB287, RRB288, RRB289 and RRB290 bound in a concentration-dependent manner to the NcfA peptide, but not to the control peptide (Fig. 1).

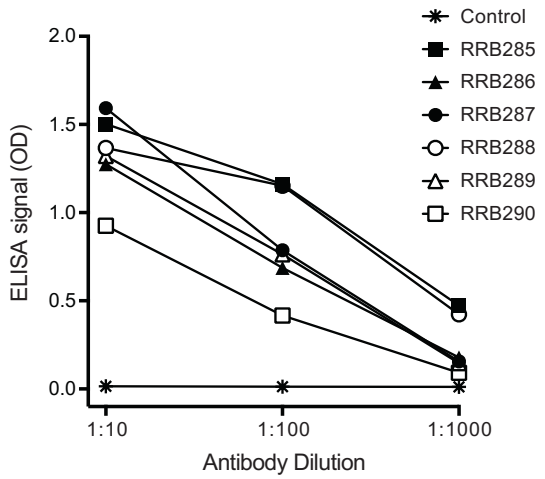

Fig. 1. Specific binding of RRB antibodies to the target NcfA peptide, as detected by ELISA. 'Control' indicates the binding of RRB285 to the negative control peptide (all other control curves were superimposed).

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

Conflict of interest

The authors declare no conflict of interest.