RB431, RB432, RB433, RB434, and RB435 antibodies recognize a Dictyostelium *RapA peptide by ELISA*

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Abstract

The recombinant antibodies RB431, RB432, RB433, RB434, and RB435 detect by ELISA a synthetic peptide from the *Dictyostelium* RapA protein.

Introduction

RapA (Ras-related protein, RAs-Proximate 1 or Rap1; DDB_G0291237, UniProt #P18613) is a Rap GTPase of the amoeba *Dictyostelium discoideum*. Here we describe the ability of five recombinant antibodies (RB431, RB432, RB433, RB434, and RB435) to detect by ELISA a synthetic biotinylated peptide from the RapA protein.

Materials & Methods

Antibodies: ABCD_RB431, ABCD_RB432, ABCD_RB433, ABCD_RB434, and ABCD_RB435 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 mouse Fc (MRB431, MRB432, MRB433, MRB434, and MRB435). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to the residues 85 to 106 of the RapA protein (SIISNSTFNELPD LREQILRVK). A N-biotinylated peptide (NLIRQINRKN PVGPPSKAKSKCALL) corresponding to the last 25 C-terminal residues of the RapA protein was used as a negative control.

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 MRB 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-mouse IgG (Bio-Rad #170-6516, 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 MRB431, MRB432, MRB433, MRB434, and MRB435 bound in a concentration-dependent manner to the RapA peptide against which they were raised, but not to the negative control peptide (Fig. 1).

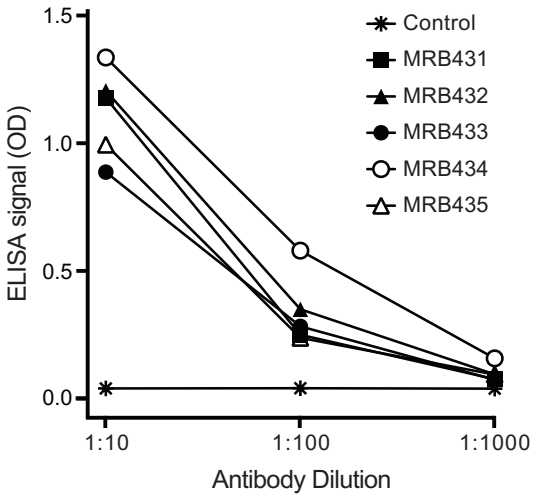

Fig. 1. Specific binding of MRB antibodies to the target RapA peptide, as detected by ELISA. 'Control' indicates the binding of MRB431 to the negative control peptide (all other control curves were superimposed).

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.