

RB388, RB389, RB390, RB391 and RB392 antibodies recognize a *Dictyostelium* AlyL peptide by ELISA

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Abstract

The recombinant antibodies RB388, RB389, RB390, RB391 and RB392 detect by ELISA a synthetic peptide from the *Dictyostelium* AlyL protein.

Introduction

AlyL (Amoeba LYsozyme Like, DDB_G0286229, UniProt #Q54M35) is a member of the amoeba lysozyme family in the amoeba *D. discoideum*. Here we describe the ability of five recombinant antibodies (RB388, RB389, RB390, RB391 and RB392) to detect by ELISA a synthetic biotinylated peptide from the AlyL protein.

Materials & Methods

Antibodies: ABCD_RB388, ABCD_RB389, ABCD_RB390, ABCD_RB391 and ABCD_RB392 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 IgG2A Fc (MRB388, MRB389, MRB390, MRB391 and MRB392). 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 23 residues (483-505) close to AlyL C-terminus (RVIST NVGPNVDIEDKIGKPIMD). A N-biotinylated peptide (ADAGSSGAQGSGSTSGDGNA) corresponding to the residues 235 to 254 of the AlyL 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 H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RB388, RB389, RB390, RB391 and RB392 bound in a concentration-dependent manner to the AlyL peptide against which they were raised, but not to the negative control peptide (Fig. 1).

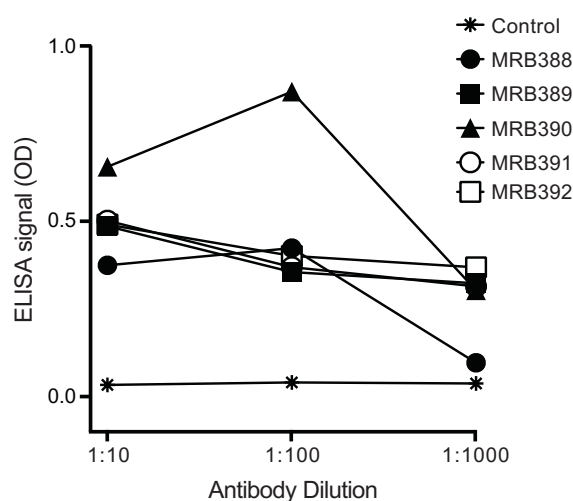


Fig. 1. Specific binding of MRB antibodies to the target AlyL peptide, as detected by ELISA. 'Control' indicates the binding of MBR388 to the negative control peptide (all other control curves were superimposed).

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.