RB376, RB377 and RB378 antibodies recognize a Dictyostelium AlyA peptide by ELISA

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Abstract

The recombinant antibodies RB376, RB377 and RB378 detect by ELISA a synthetic peptide from the *Dictyostelium* AlyA protein.

Introduction

AlyA (Amoeba LYsozyme, DDB_G0275123, UniProt #Q8T1G4) is a member of the amoeba lysozyme family in the amoeba *D. discoideum* (Muller *et al*., 2005). Here we describe the ability of three recombinant antibodies (RB376, RB377 and RB378) to detect by ELISA a synthetic biotinylated peptide from the AlyA protein.

Materials & Methods

Antibodies: ABCD_RB376, ABCD_RB377 and ABCD_RB378 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 (MRB376, MRB377 and MRB378). 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 67 to 102 of the AlyA protein (DSQRFGCGKY LNLCRSGKCVKAQIYDAGPAMWVEQD). A N-biotinylated peptide (DSRPLGPFNVTESEMAQLFIDHE IAMAQCEAEK) corresponding to the residues 141 to 173 of the AlyA 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 RB376, RB377 and RB378 bound in a concentration-dependent manner to the AlyA peptide against which they were raised, but not to the negative control peptide (Fig. 1).

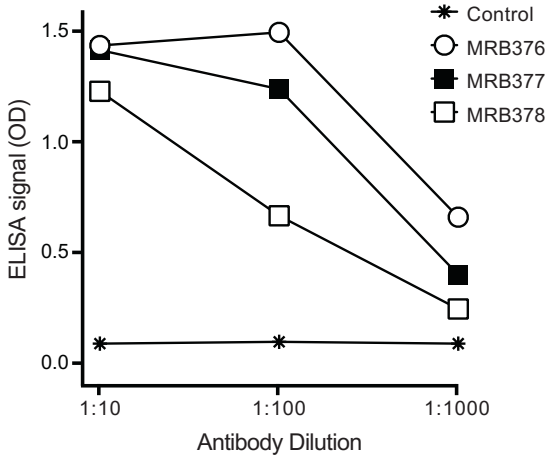

Fig. 1. Specific binding of MRB antibodies to the target AlyA peptide, as detected by ELISA. 'Control' indicates the binding of MBR376 to the negative control peptide (all other control curves were superimposed).

References

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Conflict of interest

The authors declare no conflict of interest.