RB447, RB452 and RB453 antibodies recognize a Dictyostelium AlyL protein by ELISA

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Abstract

The recombinant antibodies RB447, RB452 and RB453 detect by ELISA the *Dictyostelium* AlyL fused to a GST 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 three recombinant antibodies (RB447, RB452 and RB453) to detect by ELISA fragments of the AlyL protein fused to GST and produced in bacteria.

Materials & Methods

Antibodies: ABCD_RB447, ABCD_RB452 and ABCD_RB453 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 (MRB447, MRB452 and MRB453). 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: RB447 was raised against a GST protein fused to the residues 18 to 84 of the AlyL protein. RB452 and RB453 were raised against a GST protein fused to the C-terminal residues (534 to 572). The chimeric GST-AlyL constructs were used as antigen for ELISA detection. GST was used as negative control.

Protocol: The whole procedure was carried out at room temperature. Bacterial lysates containing GST proteins were incubated in a glutathione-coated 96-well plate (Pierce #15240) 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 RB447, RB452 and RB453 bound in a concentration-dependent manner to the GST-AlyL antigen against which they were raised, but not to the GST negative control (Fig. 1).

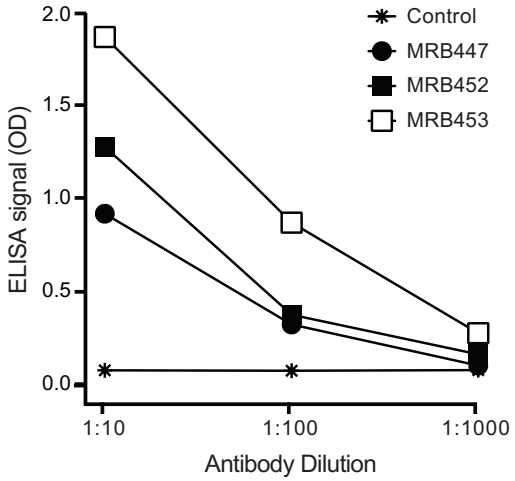

Fig. 1. Specific binding of MRB antibodies to the target GST-AlyL protein, as detected by ELISA. 'Control' indicates the binding of MRB447 to GST (all other control curves were superimposed).

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.