RB448, RB449, RB450 and RB451 antibodies recognize a peptide from the D. discoideum AlyL protein by ELISA

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Abstract

The recombinant antibodies RB448, RB449, RB450 and RB451 detect by ELISA a fragment of the D. discoideum AlyL protein 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 Dictyostelium discoideum (Muller et al., 2005). Here we describe the ability of four recombinant antibodies (RB448, RB449, RB450 and RB451) to detect by ELISA a fragment of the AlyL protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RB448, ABCD_RB449, ABCD_RB450 and ABCD_RB451 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima et al., 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc et al., 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB448, MRB449, MRB450 and MRB451). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~20-100 mg/l) were collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the residues 18-84 (QQNTCAQLCKDNKDMCCSLSKNSEYLLTTHNKEEKTQSCGDKSFSEDDYYVAGNRFGCGNSVTIC) of the D. discoideum AlyL protein. This chimeric GST-AlyL was 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 H2SO4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies MRB448, MRB449, MRB450 and MRB451 bound in a concentration-dependent manner to the GST-AlyL antigen, but not to the GST negative control (Fig. 1). Note that this antigen only encompasses a small portion of the AlyL protein, and that it is presumably not properly folded. Being produced in bacteria, it also lacks several post-translational modifications (disulfide bridges, glycosylations) typical of secreted proteins. Further experiments will be necessary to determine if and in what conditions these antibodies recognize the full AlyL protein.

Fig. 1. Specific binding of MRB antibodies to the target GST-AlyL protein, but not to GST (shown only for MRB448, all other background curves are superimposed), as detected by ELISA.

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.