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

Philippe Hammel

*Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*

## 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 (QQNTCAQLCKDNKDMCCSLSKNSEYLLTTHNKEEKTQSCGDKFNSED YYVAGSNRFGCGNSVTICKV) 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 $H_2SO_4$. 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.

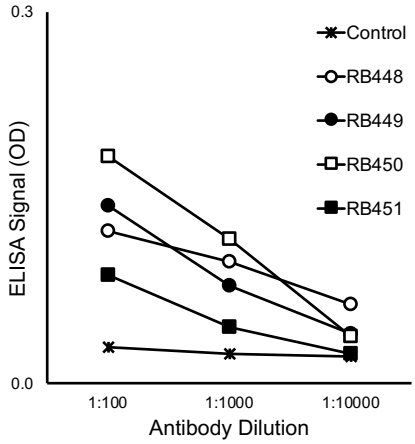


**Fig. 1.** Specific binding of MRB antibodies to the target GST-AlyL protein, but not to GST (shown only for MRB448, all other background curves are superimposed), as detected by ELISA.

## References

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## Conflict of interest

The authors declare no conflict of interest.