# *RB513, RB514, RB515, RB516, RB517 and RB518 antibodies recognize putative lysosomal proteins from Dictyostelium discoideum by ELISA*

Philippe Hammel, Cyril Guilhen

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

## Abstract

The recombinant antibodies RB513, RB514, RB515, RB516, RB517 and RB518 detect by ELISA antigens in a purified mixture of *Dictyostelium discoideum* putative lysosomal proteins.

## Introduction

*D. discoideum* lysosomes contain a vast array of degradative lysosomal enzymes. Here we describe the ability of six recombinant antibodies (RB513, RB514, RB515, RB516, RB517 and RB518) to detect by ELISA antigens present in a purified mixture of biotinylated putative lysosomal proteins.

## Materials & Methods

**Antibodies:** ABCD_RB513, ABCD_RB514, ABCD_RB515, ABCD_RB516, ABCD_RB517 and ABCD_RB518 antibodies (ABCD nomenclature, 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 (MRB513, MRB514, MRB515, MRB516, MRB517 and MRB518). 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:** In *D. discoideum*, lysosomal enzymes are frequently sulphated, and thus expected to be negatively charged even at a very acidic pH. *D. discoideum* cells were lysed at pH 3 in a 50 mM phosphate buffer containing 0.5% Triton X100 and a cocktail of protease inhibitors (Leupeptin, Aprotinin and PMSF). The suspension was centrifuged (30'000 g during 10 minutes at 4 °C) and the supernatant was collected and incubated for one hour with an anionic exchange resin (Q Sepharose® GE Healthcare #GE17-0510-10). The resin was washed 5 times and putative lysosomal proteins were eluted using the lysis buffer supplemented with 0.5M of NaCl. Antibodies were raised against the lysosomal proteins biotinylated using EZ-Link® Micro Sulfo-NHS-Biotinylation Kit (Thermo Scientific #21925). As a negative control, a mix of biotinylated BSA and keratin was used.

**Protocol:** The whole procedure was carried out at room temperature. Biotinylated lysosomal proteins at saturating concentration (5 µg/ml) 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. After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-16516, dilution 1:1'000, 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 MRB513, MRB514, MRB515, MRB516, MRB517 and MRB518 bound in a concentration-dependent manner to the putative lysosomal proteins against which they were raised, but not to the negative control proteins (Fig. 1).

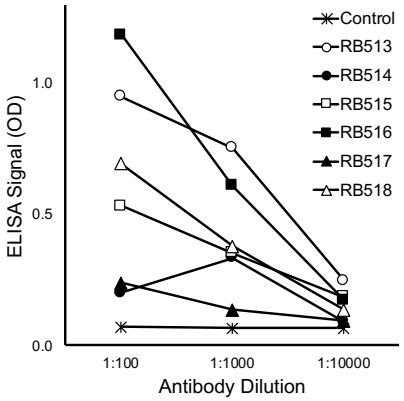


**Fig. 1.** Specific binding of MRB antibodies to the target lysosomal proteins, as detected by ELISA. 'Control' indicates the binding of MRB516 to the negative control proteins (all other control curves were superimposed).

## 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.