Antibodies ABCD_RB989-RB998 recognize the Dictyostelium discoideum GAA protein by ELISA

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Abstract

The 10 recombinant antibodies ABCD_RB989 to ABCD_RB998 detect by ELISA the purified *Dictyostelium discoideum* GAA protein.

Introduction

The *Dictyostelium discoideum* GAA protein (UniProt #Q55D50) is a putative alpha-glucosidase, orthologous to the human lysosomal alpha-glucosidase (UniProt #P10253, Persson *et al*., 2023), which is essential for glycogen degradation in lysosomes (Hermans *et al*., 2004). Here we describe the ability of ten recombinant antibodies (ABCD_RB989-RB998) to detect by ELISA a purified Twin Strep-Tagged GAA protein from *D. discoideum*.

Materials & Methods

Antibodies: ABCD_RB989 to ABCD_RB998 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/, referred to collectively as RB989-998) were discovered by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/). Briefly, a synthetic VHH phage display library (in-house) was panned against a Twin Strep-tagged GAA protein (see antigen section). After three rounds of panning, selected phage vectors were isolated using a plasmid preparation kit (Qiagen), and the VHH inserts were subcloned into custom-made expression vectors and sequenced. The selected antibodies were produced as minibodies with the antigen-binding VHH fused to a rabbit IgG Fc. HEK293 suspension cells growing in HEK TF medium (Sartorius #861-0001), supplemented with 0.1% Pluronic F68 (Sigma #P1300) were transiently transfected with the vectors coding for each VHH-Fc. Supernatants were collected after 3 days. Estimated production yields were approximately 100-110 mg/L for antibodies RB989, RB995, RB997 and RB998, and between 70-80 mg/L for RB990, RB991, RB992, RB993, RB994 and RB996.

Antigen: We used a fusion protein composed of a human IL2 signal sequence for insertion in the ER followed by the coding sequence of the *D. discoideum* GAA protein without sequence signal (amino acids 24 to 867) and fused at its C-terminus to a Twin-Strep-Tag® (IBA Lifesciences, GAA-TST). The fusion protein was produced in transiently transfected HEK293 cells and purified using MagStrep Streptactin XT beads according to the manufacturer instruction (IBA Lifesciences #2-5090-002). A TST tagged PldX protein from *D. discoideum* (PldX-TST, UniProt #Q55CF8, residues 20-427) was produced the same way and used as a negative control.

Protocol: The whole ELISA procedure was carried out at room temperature. Purified TST tagged proteins were coated directly on Maxisorp Elisa plates (Nunc #44-2404-21) at 3 µg/ml in PBS for 45 min. As a saturation agent, 50 µL of PBS-BSA 3% (w/v) was added, followed by 20 minutes of incubation. Each well was then 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 RB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times with 100 µL of washing buffer, wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma #A8275, 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 for background correction.

Results and Discussion

Antibodies RB989–RB998 bound to the GAA-TST antigen in a concentration-dependent manner but did not bind to the PldX-TST negative control (Fig. 1).

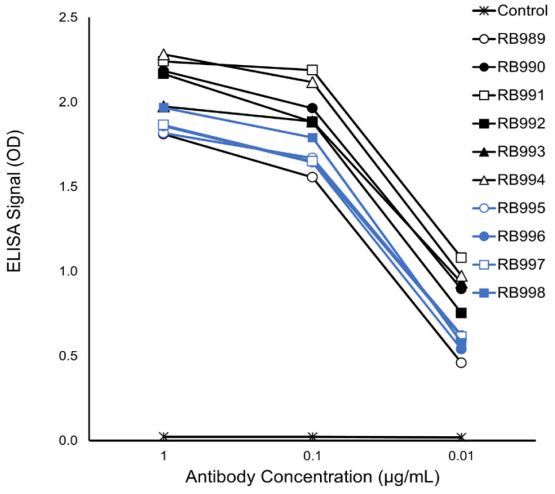

Fig. 1. Specific binding of RB antibodies to the target GAA-TST protein, but not to the negative control (shown only for RB989; other background curves are superimposed), as detected by ELISA.

References

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Conflict of interest

Philippe Hammel is a cofounder and shareholder of ABCD antibodies SA.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.