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Reference
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Abstract
The recombinant antibodies RB464, RB465, RB466 and RB467 do not detect by Western blot the full-length AlyA protein from Dictyostelium discoideum.

Introduction
AlyA (Amoeba LYsozyme, DDB_G0275123, UniProt #Q8T1G4) is a member of the amoeba lysozyme family in the amoeba D. discoideum (Muller et al., 2005). Here we describe the inability of four recombinant antibodies (RB464, RB465, RB466 and RB467) to detect the full-length AlyA protein by Western blot.

Materials & Methods
Antibodies: RB464, RB465, RB466 and RB467 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 fused to a rabbit Fc (RRB464, RRB465, RRB466 and RRB467). 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. As a positive control, the anti-6xHis antibody AD946 (Lamrabet and Jauslin, 2018) was used.

Antigen: RB464, RB465, RB466 and RB467 were raised against a N-biotinylated synthetic peptide corresponding to 43 residues close to the AlyA C-terminus (LTDSRPLGPNFNTESEQELFIDHEIAMAQCEAEKTCNGFDL). D. discoideum DH1 (WT) cells expressing a 6xHis-tagged AlyA protein (AlyA-His, 6xHis-tag fused to the C-terminus) were used to detect the full-length AlyA protein.

Protocol: 5x10⁶ D. discoideum cells were pelleted and resuspended in 200 µL of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 20 µL of each sample was migrated (200 V, 30 min) in a 4-15% acrylamide gel (MiniPROTEAN® TGX™ Precast Gel, Biorad #456-1086), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked during 1 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed three times for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (dilution 1:2 in PBS-Tween), overnight at 4 °C, then washed three times for 15 minutes. The membranes were then incubated with horseradish peroxidase-coupled goat anti-rabbit or anti-mouse IgG (Biorad #170-6515 and #170-6516, respectively; dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (K-12043, Advansta Corporation) using a PXi-4 gel imaging systems (Syngene).

Results
Antibodies RRB464, RRB465, RRB466 and RRB467 did not specifically recognize the endogenous AlyA protein, nor the overexpressed 6xHis-tagged AlyA protein in WT cells (Fig. 1). The tagged protein was detected in the AlyA-His expressing cells with an anti-6xHis antibody (AD946).

Fig. 1. No specific binding of RRB antibodies to cells overexpressing AlyA-His. AlyA-His was successfully detected by the anti-6xHis AD946 antibody (position indicated by an asterisk), but not by any of the RRB antibodies tested here.

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Conflict of interest
The authors declare no conflict of interest.