AF394 and AF395 antibodies recognize a GFP tagged recombinant protein by Western blot

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Abstract

The recombinant antibodies AF394 and AF395 detect by Western blot the GFP-AlyL protein from *Dictyostelium discoideum*. AF396 does not.

Introduction

Green Fluorescent Protein (GFP) is one of the most widely-used fluorescent reporter proteins. Here we describe the ability of two recombinant antibodies (AF394 and AF395) to detect the GFP-tagged AlyL (Amoeba LYsozyme Like, DDB_G0286229, UniProt #Q54M35) protein from *D. discoideum* by Western blot.

Materials & Methods

Antibodies: ABCD_AF394, ABCD_AF395 and ABCD_AF396 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the monoclonal anti-GFP clones GBP1, GBP4 (Kirchhofer *et al.*, 2010) and VHH (Rothbauer *et al*., 2006), respectively, joined by a peptide linker $(GGGGS)_3$. 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-AlyL MRB447 antibody (Lamrabet and Jauslin, 2018) was used to detect the AlyL protein.

Antigen: *D. discoideum* DH1 (WT) cells expressing a GFP-tagged AlyL protein (GFP fused to the C-terminus of AlyL) were used to detect the GFP protein.

Protocol: $5x10^6$ *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); samples were not boiled. 20 µL of each sample was migrated (200 V, 30 min) in a 4-15% acrylamide gel (Mini-PROTEAN® 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 2 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 1:10 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-mouse IgG (Biorad #170-6516, 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 AF394 and AF395 (but not AF396) specifically recognize the GFP-moiety in the GFP-AlyL (Fig. 1). The tagged protein was also detected with an anti-AlyL antibody (MRB447).

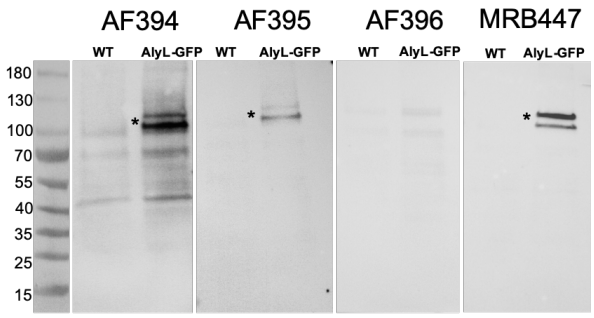

Fig. 1. Specific binding of GFP antibodies to WT cells overexpressing AlyL-GFP. AlyL-GFP was successfully detected by AF394 and AF395 and by the anti-AlyL MRB447 antibody (positions indicated by asterisks), but not by AF396. The endogenous AlyL protein was not detected in non-transfected WT cells.

References

Kirchhofer A, Helma J, Schmidthals K, *et al*. Modulation of protein properties in living cells using nanobodies. Nat Struct Mol Biol. 2010; 17:133-8. PMID:20010839

Lamrabet O, Jauslin T. RB447, RB448, RB449, RB450, RB451 and RB453 antibodies recognize the Dictyostelium AlyL protein by Western Blot. Antibody Reports. 2018; 1:e02. doi:10.13097/journals/abrep.2018.e2

Rothbauer U, Zolghadr K, Tillib S, *et al*. Targeting and tracing antigens in live cells with fluorescent nanobodies. Nat Methods. 2006; 3:887-9. PMID:17060912

Conflict of interest

The authors declare no conflict of interest.