The AL626 antibody recognizes by western blot an ALFA-tagged protein produced in *Dictyostelium discoideum*

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Abstract

The AL626 antibody against the ALFA tag detects by western blot an ALFA-tagged protein produced in the amoeba *Dictyostelium discoideum*.

Introduction

The ALFA tag is a new, rationally designed peptide tag to detect and purify tagged proteins (Götzke et al., 2019). Here we describe the ability of the AL626 recombinant antibody, derived from the NbALFA nanobody (Götzke et al., 2019), to detect an ALFA-tagged amoeba protein produced in *Dictyostelium discoideum* by western blot.

Materials & Methods

Antibodies: The ABCD_AL626 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding VHH fused to a mouse IgG2A Fc. The synthesized VHH sequence (GeneArt, Invitrogen) corresponds to the sequences of the variable region of the synthetic camelid antibody NbALFA (Götzke et al., 2019). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the VHH-Fc. Supernatant (100 mg/L) was collected after 4 days.

Antigen: *Dictyostelium discoideum* cells (strain DH1) growing in HL5 medium, transfected with either a C-terminally or a N-terminally ALFA-tagged protein (Uniprot #Q86LA4), were used to detect the tag (`SRLEEELRRRLTEP`). For the N-terminal version, the tag coding sequence was placed after the signal sequence. Non-transfected *Dictyostelium discoideum* cells were used as a control.

Protocol: $5x10^5$ transfected *Dictyostelium discoideum* cells were pelleted and resuspended in 20 µL of 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). Each sample was migrated (200 V, 30 min) in a 4-20% acrylamide gel (SurePAGE Bis-Tris, Genscript #M00655), 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 5 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with the primary antibody AL626 (dilution 1:50 in PBS-Tween + 7% (w/v) milk) for 1 hour at room temperature and washed three times for 5 minutes. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170- 6516, dilution 1:3000) and washed twice for 5 minutes and once for 15 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences, #WBLUC0500) using a PXi-4 gel imaging systems (Syngene).

Results

The AL626 antibody specifically recognizes the ALFA-tagged amoeba protein (tag positioned either at the C- or N-terminus). No signal was detected in non-transfected *Dictyostelium discoideum* cells (Fig. 1). The difference between the predicted molecular mass (22 kDa) and the observed ~28 kDa is presumably due to the glycosylation of the amoeba protein (Uniprot #Q86LA4) in the secretory pathway.

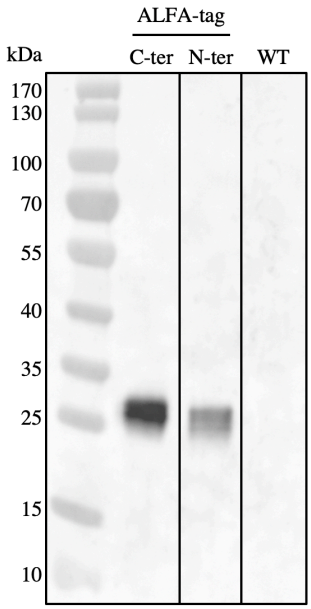

Fig. 1. Specific binding of the AL626 antibody to the ALFA-tagged amoeba protein (predicted molecular mass: 22 kDa). The ALFA-tag was placed either at the C-terminal (C-ter) or N-terminal (N-ter) end. No band was observed in non-transfected *Dictyostelium discoideum* cells.

References

Götzke H, Kilisch M, Martínez-Carranza M, *et al*. The ALFA-tag is a highly versatile tool for nanobody-based bioscience applications. Nat Commun. 2019; 10(1):4403. PMID:31562305

Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. Nucleic Acids Res. 2020; 48(D1):D261-D264. PMID: 31410491

Conflict of interest

The authors declare no conflict of interest.