The AL626 antibody recognizes an ALFA-tagged protein by western blot

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Abstract

The AL626 antibody against the ALFA tag detects an ALFA-tagged human TAC protein by western blot.

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 human TAC protein by western blot.

Materials & Methods

Antibodies: The ABCD_AL626 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) 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: HEK293 cells (growing in DMEM GlutaMAX™ [Gibco, #31966] supplemented with 8% Fetal Bovine Serum [Gibco, #10270]), transiently transfected 2 days before with a C-terminally ALFA-tagged TAC protein (Uniprot #P01589), were used to detect the tag (`PSRLEEELRRRLTE`). Cells expressing the RB376 antibody with a mouse Fc portion were used as a control (Lamrabet, 2019).

Protocol: $1x10^6$ HEK293 cells were pelleted and resuspended in 20 µL of lysis buffer (PBS + 0.5% Triton + aprotinin 10 µg/ml + leupeptin 20 µg/ml + iodoacetamide 1.8 mg/ml + PMSF 18 µg/ml) for 15 min at 4°C, then samples were centrifuged (10,000 g) for 15 min at 4°C and the supernatants collected. 10 µL of each sample was resuspended in reducing or non-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 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, #M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB23001). 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 incubated with AL626 (dilution 1:50 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) (Amersham Biosciences, #WBLUC0500) using a PXi-4 gel imaging systems (Syngene).

Results

The AL626 antibody specifically recognizes the ALFA-tagged TAC protein; the mature, 55 kDa TAC isoform can be seen, along with the 48 kDa precursor and minor cleaved forms (Fig. 1). HEK cells transfected with an unrelated construct (RB376 antibody with a mouse IgG fragment) were used as a control (Fig. 1, line C): the bands correspond to the detection of the RB376 monomer (~45kDa) and dimer (~90kDa) by the secondary anti-mouse IgG antibody.

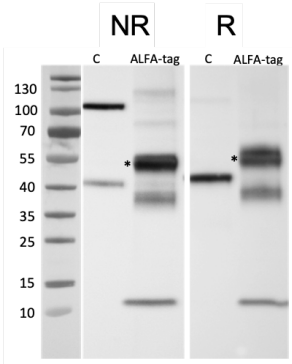

Fig. 1. Specific binding of the AL626 antibody to the ALFA-tagged TAC protein (positions indicated by asterisks) (predicted molecular mass: ~55kD in reducing conditions). No difference was observed between non-reducing (NR) and reducing (R) condition.

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

Lamrabet O. RB376 and RB377 antibodies recognize the Dictyostelium AlyA protein by Western blot. Antib. Rep. 2019; 2:e10. doi:10.24450/journals/abrep.2019.e10

Conflict of interest

The authors declare no conflict of interest.