AFXXX and ACXXX (Antibodies identifiers from ABCD database, e.g. AG513) antibodies recognize (name of the target) by western blot

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Abstract

The recombinant antibodies AFXXX and ACXXX (ABCD identifiers of the antibodies) detect by western blot (name of the target).

Introduction (3-15 lines)

Short description of the target. When available, please specify an identifier for the target (e.g: UniProt #P06213). Here, we describe the ability of (ABCD identifiers of the antibodies) to detect (name of the target) by western blot.

Materials & Methods

**Antibodies:** ABCD\_AFXXX and ABCD\_ACXXX, antibodies (ABCD nomenclature, <http://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (<http://unige.ch/medecine/antibodies/>) and produced as minibodies/nanobodies with the antigen-binding scFv (or VHH in the case of nanobodies) portion fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker (GGGGS)3 . HEK293 suspension cells growing in HEK TF medium (Xell #861-0001, Sartorius), supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vector coding for the VHH/scFv-Fc of each antibody. Supernatants (~50-80 mg/L) were collected after 4 days.

**Antigen (3-5 lines):** Description of the antigen used (cell lysate, transfected cell lysate / supernatant, purified protein,…)

**Protocol:** The recombinant humanMOG-Fc-Avi-6xHis protein was purified from HEK293 cells (560 ng/µL) and diluted in 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) -mercaptoethanol). Three different amounts of the protein were used in this study (225, 22.5 and 2.25 ng). 20 µL of each sample was migrated (200 V, 30 min) on 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 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 incubated with AD946 (dilution 1:2 in PBS-Tween) overnight at 4°C, then washed three times for 15 minutes. The membranes were then incubated 1 hour with horseradish peroxidase-coupled goat anti-rabbit IgG (Biorad #REF, 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) using a PXi-4 gel imaging systems (Syngene).

Results & Discussion

Antibodies AFXXX and ACXXX detect (name of the target )in sample (Fig. 1).

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**Fig. 1.** Specific binding of antibodies to name of the target, as detected by western blot. Short description of the figure.

References

Add any needed references in the APA format

Ex:

Rougeaux H, Kervarec N, Pichon R, Guezennec J. Structure of the exopolysaccharide of *Vibrio diabolicus* isolated from a deep-sea hydrothermal vent. Carbohydr Res. 1999 Nov 23;322(1-2):40-5. PMID: 10629947.

Conflict of interest

The authors declare no conflict of interest.

**Data Availability Statement**

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