AFXXX and AFXXX(Antibody identifiers from ABCD database, e.g. AG513) antibodies recognize (name of the target) by immunofluorescence

1Author, 2Author

*1Affilitation
 2 Affilitation*

Abstract

The recombinant antibodies AFXXX and ACXXX (ABCD identifiers of the antibodies) detect by immunofluorescence (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 immunofluorescence.

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 (endogenous protein, transfected cells, …)

**Protocol:** The whole procedure was carried out at room temperature. Transfected cells were rinsed once with PBS, fixed with PBS + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 10 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the antibody-containing supernatants (non-diluted). After 3 washes (5 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes, #A11029). After 3 washes (5 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results & Discussion

Antibodies AFXXX and ACXXX detect (name of the target) in (cell type). No signal was observed in the negative control (Fig. 1).

Insert figure (jpeg, png, 300 dpi ; 80 mm-wide, fonts and style: Arial 10 points)

**Fig. 1.** Specific binding of antibodies to name of the target, as detected by immunofluorescence. 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.