

# AF397, AK652, AN193 and AV442 antibodies recognize a GFP-tagged protein by western blot

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## Abstract

AF397, AK652, AN193 and AV442 antibodies against the GFP protein recognize a GFP-tagged human TAC protein by western blot.

## Introduction

The green fluorescent protein (GFP) (Uniprot P42212) is a protein tag originally isolated from the jellyfish *Aequorea victoria*, widely used as a fluorescent reporter to detect and visualize GFP-fused proteins (Tsien, 1998). The AF397, AK652, AN193 and AV442 recombinant antibodies detect a GFP-tagged human TAC/IL2RA protein by western blot.

## Materials & Methods

**Antibodies:** The anti-GFP ABCD\_AF397, ABCD\_AK652, ABCD\_AN193 and ABCD\_AV442 antibodies (<https://web.expasy.org/abcd/>, ABCD nomenclature) were produced by the Geneva Antibody Facility (<https://www.unige.ch/medecine/antibodies/>) as mini-antibodies with the antigen-binding domain fused to a rabbit IgG Fc. The synthesized antibody sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker (GGGGS)<sub>3</sub> (Table 1). HEK293 suspension cells (growing in HEK TF medium, Xell 861-0001, supplemented with 0.1% Pluronic F68, Sigma P1300) were transiently transfected with the vector coding for the mini-antibodies. Supernatants (see Table 1 for individual yields) were collected after 4 days.

The recombinant antibody AJ519, which recognizes human TAC/IL2RA protein (Uniprot P01589), was used as a positive control (Arsimoles *et al.*, 2020).

**Table 1:** Clone number, epitope, reference and production yields for the antibodies used in this study.

ABCD	Clone	Binder type	Reference	Yield (mg/L)
AF397	LaG-2	VHH	Fridy <i>et al.</i> , 2014	140
AK652	BH-GBP2	VHH	Pellis <i>et al.</i> , 2012	120
AN193	3G86.32	DARPin	Brauchle <i>et al.</i> , 2014	50
AV442	N86/44.1	scFv	Andrews <i>et al.</i> , 2019	120
AJ519	7G7	scFv	Arsimoles <i>et al.</i> , 2020	40

**Antigen:** HEK cells (growing in DMEM GlutaMAX™, Gibco 31966; supplemented with 8% Fetal Bovine Serum, Gibco 10270), transiently transfected 2 days before the experiment with a C-terminally GFP-tagged human TAC/IL2RA protein (Uniprot P01589), were used to detect the protein tag.

**Protocol:** 5x10<sup>6</sup> cells were pelleted and lysed for 15 min in 100 µL of ice-cold lysis buffer (25 mM Tris-HCl pH 7.4 + 0.5 % Triton X-100 + 120 mM NaCl) containing protease inhibitors. Lysate was centrifuged 15 min, 10'000 g at 4 °C to remove nuclei. One volume of sample buffer was added to the lysate (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS). Dilutions of each sample (500'000 and 100'000 cells) were migrated (150 V, 45 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen IB23001). The membranes were blocked during 60 min in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed once for 15 minutes in PBS + 0.1% (v/v) Tween20 (PBS-Tween). The membranes were then incubated overnight at RT with the primary antibodies (final concentration 5 mg/L in PBS-Tween). The membranes were then washed three times (15+15+10 min) in PBS-Tween, incubated for 1 hour with the horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich A8275, dilution 1:3000) and washed three times (15 min) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

## Results

AF397, AK652, AN193 and AV442 antibodies specifically recognized GFP in cells transfected with the GFP-tagged TAC/IL2RA protein, detecting two bands between 60 and 70 kDa (corresponding to immature and mature glycosylation forms, respectively; Fig. 1). The same bands were seen with AJ519, an anti-TAC recombinant antibody used as positive control (Fig. 1).

In addition, anti-GFP antibodies detected a smaller protein (37 kDa) which presumably corresponds to a proteolytically processed form of the IL2RA-GFP protein (Fig. 1).

The specificity of the signal was verified by the absence of anti-GFP staining in non-transfected cells (Fig. 1, "NT").

## References

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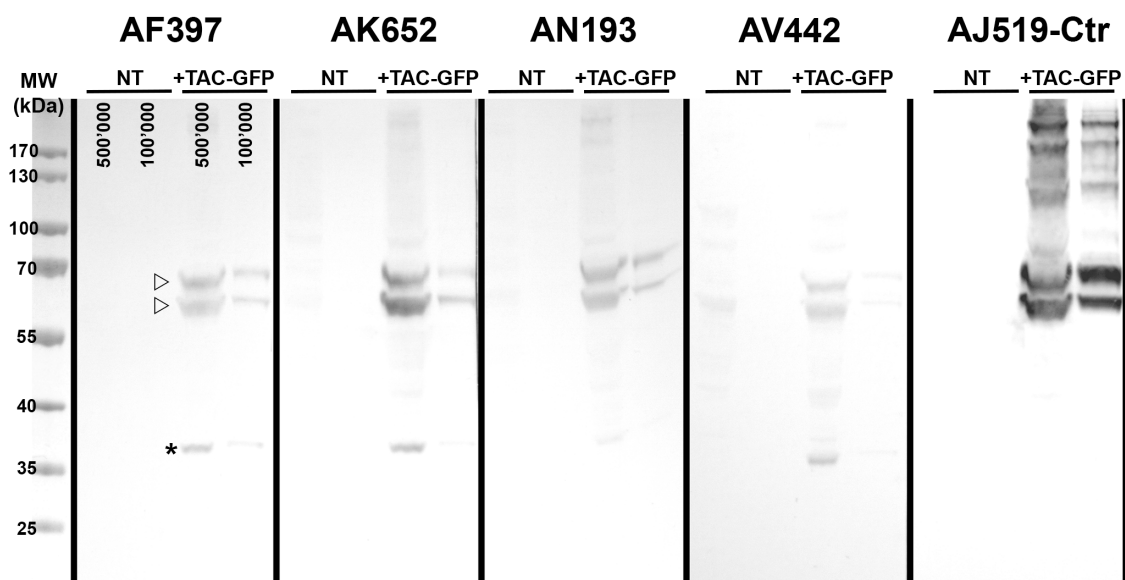
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## Conflict of interest

The authors declare no conflict of interest.



**Fig. 1.** AF397, AK652, AN193 and AV442 specifically recognize GFP in HEK cells expressing a GFP-tagged TAC/IL2RA protein (“+TAC-GFP”). Two bands, indicated by arrowheads (shown only for AF397), correspond to different glycosylation states of the IL2RA protein. The same bands are seen for the positive control, labeled with the anti-TAC AJ519 antibody (“AJ519-Ctr”). The anti-GFP antibodies also detect free GFP (around 35 kDa, asterisk). No labelling was seen in non-transfected cells (“NT”). For each antibody and condition, two sample dilutions were used: 500'000 and 100'000 cells/well (labels shown only for AF397).