

# The AJ519 antibody detects the human TAC/ILR2A protein by flow cytometry

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## Abstract

The AJ519 antibody detects the human TAC protein by flow cytometry.

## Introduction

The alpha subunit of the interleukin 2 receptor, also known as the TAC antigen (Uniprot #P01589), is a protein displayed notably at the surface of T lymphocytes (Uchiyama *et al.*, 1981; Malek and Castro, 2010). Here, we describe the ability of the AJ519 antibody, a single chain fragment (scFv) derived from the 7G7 hybridoma, to successfully detect the TAC protein by flow cytometry in TAC-transfected HEK293 cells.

## Materials & Methods

**Antibodies:** ABCD\_AJ519 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) and IgG produced by 7G7 hybridoma were produced by the Geneva Antibody Facility (www.unige.ch/antibodies/). AJ519 antibody was produced as mini-antibody with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the 7G7 hybridoma (Rubin *et al.*, 1985) joined by a peptide linker (GGGS)<sub>3</sub>. The sequencing of the 7G7 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in serum-free FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. AJ519 supernatant (50 mg/L) was collected after 4 days.

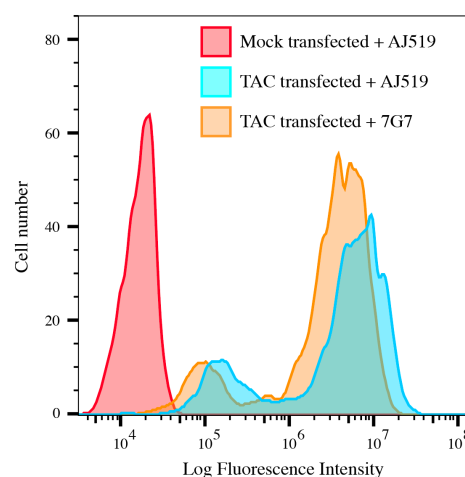
**Antigen:** The 7G7 hybridoma was originally raised against human influenza virus-stimulated PBMC in BALB/cJ mice (Rubin *et al.*, 1985). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected 3 days before the experiment with the vector coding for the full-length human TAC protein.

**Protocol:** The whole procedure was carried out at 4°C. 500'000 transfected cells were pelleted and washed once with washing buffer (PBS + 0.2% BSA (w/v)). Cells were then incubated for 20 minutes with either the original mouse hybridoma 7G7 supernatant (dilution 1:2 in PBS-BSA) or with the reformatted scFv antibody AJ519 (5 mg/L). After two washes in washing buffer, cells were incubated for 20 minutes with either secondary goat anti-mouse or anti-rabbit IgG conjugated to Alexa Fluor 488 (dilution 1:400, Molecular Probes #A11029 and #A11034

respectively). After two washes in washing buffer, cells were resuspended in 500 µL of washing buffer and analyzed with a flow cytometer (BD Accuri™ C6).

## Results

Both the IgG produced by the mouse hybridoma 7G7 and the reformatted scFv AJ519 detect the TAC protein at the cell surface of HEK293 transfected cells. No signal was detected in mock transfected cells (Fig. 1).



**Fig.1.** Both IgG produced by the mouse hybridoma 7G7 (orange) and the reformatted scFv AJ519 (blue) label HEK293 cells overexpressing the TAC protein. No signal was detected in mock transfected cells incubated with the AJ519 antibody (red).

## References

- Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. *Nucleic Acids Res.* 2019; pii:gkz714. PMID: 31410491
- Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. *Immunity.* 2010; 33(2):153-65. PMID: 20732639
- Rubin LA, Kurman CC, Biddison WE, Goldman ND, Nelson DL. A monoclonal antibody 7G7/B6, binds to an epitope on the human interleukin-2 (IL-2) receptor that is distinct from that recognized by IL-2 or anti-Tac. *Hybridoma.* 1985; 4(2):91-102. PMID:2408992.
- Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J Immunol.* 1981; 126(4):1393-7. PMID:6970774.

## Conflict of interest

The authors declare no conflict of interest.