

AI239 and RB95 antibodies recognize a GST-tagged recombinant protein by immunofluorescence

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Abstract

AI239 and RB95 antibodies against the Glutathione S-transferase (GST) protein recognize a GST-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells; AF209, AF212 and RB94 do not.

Introduction

Glutathione S-transferase (GST) (Uniprot #P08515) is a large (~215 aa) protein tag, from *Schistosoma japonicum*, used to affinity-purify GST-fused proteins, given its ability to bind its glutathione substrate with high affinity (Smith and Johnson, 1988). Here, we show that the AI239 and RB95 recombinant antibodies detect a GST-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods

Antibodies: ABCD_AF209, ABCD_AF212, ABCD_AI239, ABCD_RB094, and ABCD_RB095 antibodies (ABCD nomenclature, web.expasy.org/abcd; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the clones VHii, VHG64 (for AF209 and AF212; O'Brien *et al.*, 1999), and VHH (for AI239, Lin *et al.*, 2018) joined by a peptide linker (GGGS)₃. RB94 and RB95 were originally selected against a GST fusion protein (Blanc *et al.*, 2014); MRB94 and MRB95 are the mouse version of these antibodies. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (for MRB94, MRB95, AI239, ~100 mg/L) were collected after 4 days; AF209 and AF212 have a low production yield in this system (<5 mg/L).

Antigen: HeLa cells (growing in DMEM GlutaMAX™, Gibco #31966; supplemented with 8% Fetal Bovine Serum, Gibco #10270) cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and transiently transfected 2 days before the experiment with a C-terminally GST-tagged TAC protein (Uniprot #P01589), were used to detect the protein tag. An antibody detecting the N-terminal extracellular domain of the TAC protein (AJ519, with rabbit IgG Fc; Arsimoles *et al.*, 2020) was used as a positive control. The GST-tagged TAC protein is expected to be mostly present at the cell surface.

Protocol: The whole procedure was carried out at room temperature. Transfected HeLa 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 3 min, incubated in PBS + 0.2% (w/v) BSA (PBS-BSA) for 30 min, and then with the tested anti-GST antibodies (final concentration 5 mg/L in PBS-BSA) for 1 h. When indicated, the AJ519 antibody was added to this incubation (final concentration 2.5 mg/L in PBS-BSA). After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-647 (1:300, Molecular Probes, #A21235) and, when AJ519 was used for co-localization, goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes #A11034). After 3 washes (10 min) with PBS-BSA, cells were incubated during 10 min with DAPI (1:500, Molecular Probes, #D1306), washed twice with PBS-BSA and once with PBS, and mounted on slides (Menzel-Gläser, 76x26 mm) with Mowiol (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

AI239 and MRB95 antibodies specifically detected a signal at the plasma membrane in cells transfected with the GST-tagged TAC protein (Fig. 1A). The signal co-localized with the signal generated by the anti-TAC AJ519 antibody (Fig. 1A, arrows); the specificity of the signal was further verified by the absence of both anti-TAC and anti-GST stainings in the few non-transfected cells (Fig. 1A, arrowheads). MRB95 also labeled some cells not stained by AJ519 (Fig. 1A, pinheads). No signal was seen with AF212 and MRB94 antibodies; AF209 detected a weak signal, but the staining pattern does not correspond to the expected cell surface localization (Fig. 1B). No staining was observed when the primary antibody was omitted (Fig. 1A, No Ab).

Conflict of interest

The authors declare no conflict of interest.

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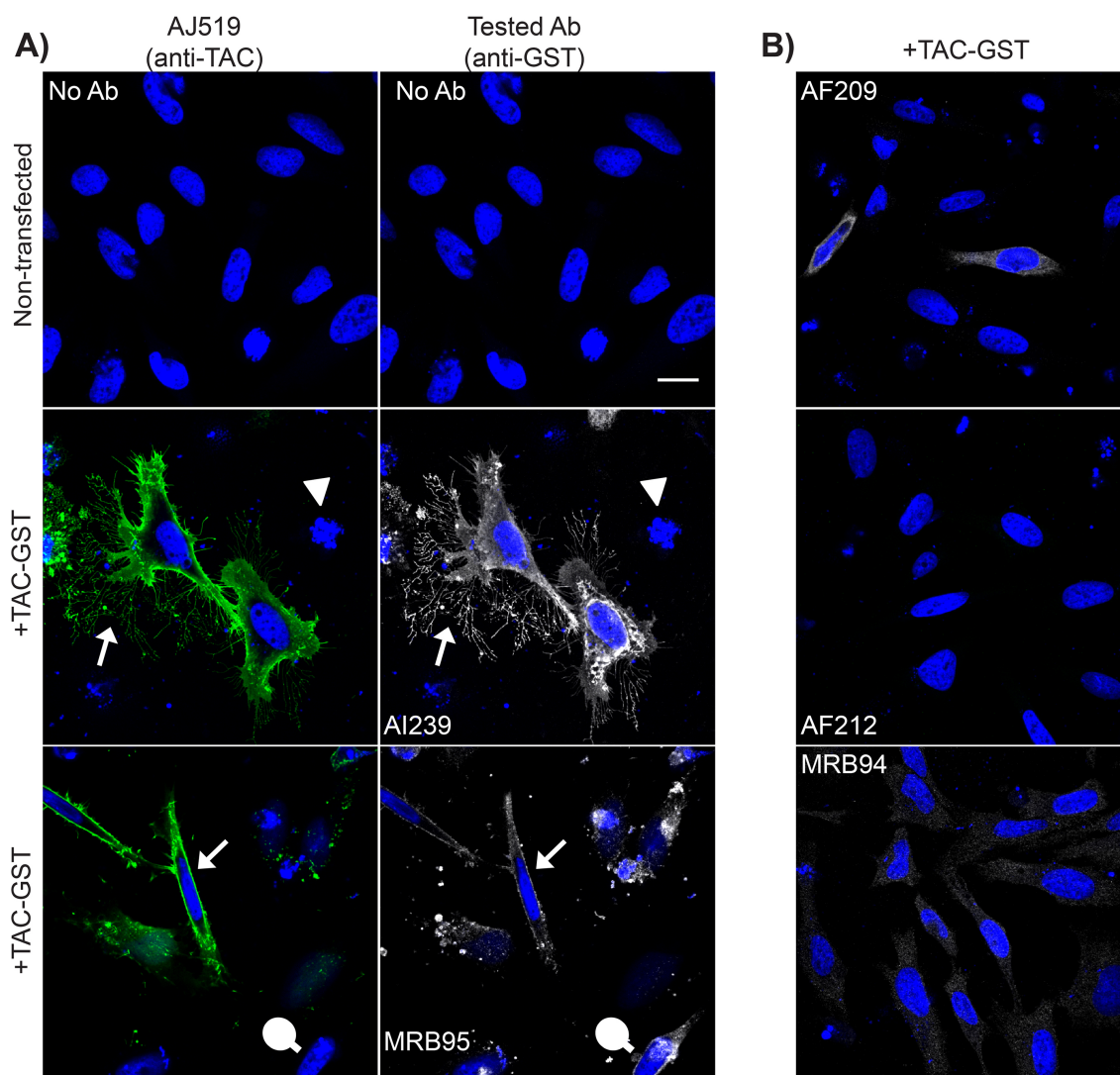


Fig. 1. (A) AI239 and MRB95 labeled the plasma membrane of HeLa cells expressing the GST-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the anti-TAC AJ519 antibody (in green); in blue, nuclei were stained with DAPI. MRB95 also labeled some cells not stained by AJ519 (pinheads). No labelling was seen when the primary antibody was omitted, or in non-transfected cells (arrowheads). **(B)** No labelling was seen for AF212 and MRB95; a faint labelling was seen for AF209, but not at the expected location. Scale bar: 20 μ m.