AE391 and AF291 antibodies recognize an HA-tagged recombinant protein by immunofluorescence

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Abstract

AE391 and AF291 antibodies against the HA tag recognize an HA-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells.

Introduction

The HA tag is a short peptide derived from the influenza virus hemagglutinin protein (Uniprot #P03435), extensively used for detection and purification of tagged proteins with anti-HA antibodies (Green *at al.*, 1982). Here, we show that the AE391 and AF291 recombinant antibodies detect an HA-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods

Antibodies: ABCD_AE391 and ABCD_AF291 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 26/9 (for AE391; Churchill *et al.*, 1994) and 12CA5 (for AF291; Arimori *et al.*, 2017) joined by a peptide linker $(GGGS)_3$. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (10 and 70 mg/L for AE391 and AF291, respectively) were collected after 4 days.

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 an HA-tagged TAC protein (Uniprot #P01589), were used to detect the peptide tag. The HA epitope sequence used was `YPYDVPDYASLRS` and it was present in the C-terminal cytosolic domain of the fusion protein. 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 HA-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 (NH_4Cl) (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-HA antibodies (final concentration 5 mg/L in PBS-BSA) and AJ519 antibody (final concentration 2.5 mg/L in PBS-BSA) for 1 h. 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 and anti-rabbit IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes, #A21235 and #A11034, respectively). 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 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

AE391 and AF291 antibodies specifically detected a signal at the plasma membrane in cells transfected with the HA-tagged TAC protein (Fig. 1). The signal co-localized with the signal generated by the anti-TAC AJ519 antibody (Fig. 1, arrows); the specificity of the signal was further verified by the absence of both anti-TAC and anti-HA stainings in the few non-transfected cells (Fig. 1, arrowheads). No staining was observed when the primary antibody was omitted (Fig. 1, No Ab).

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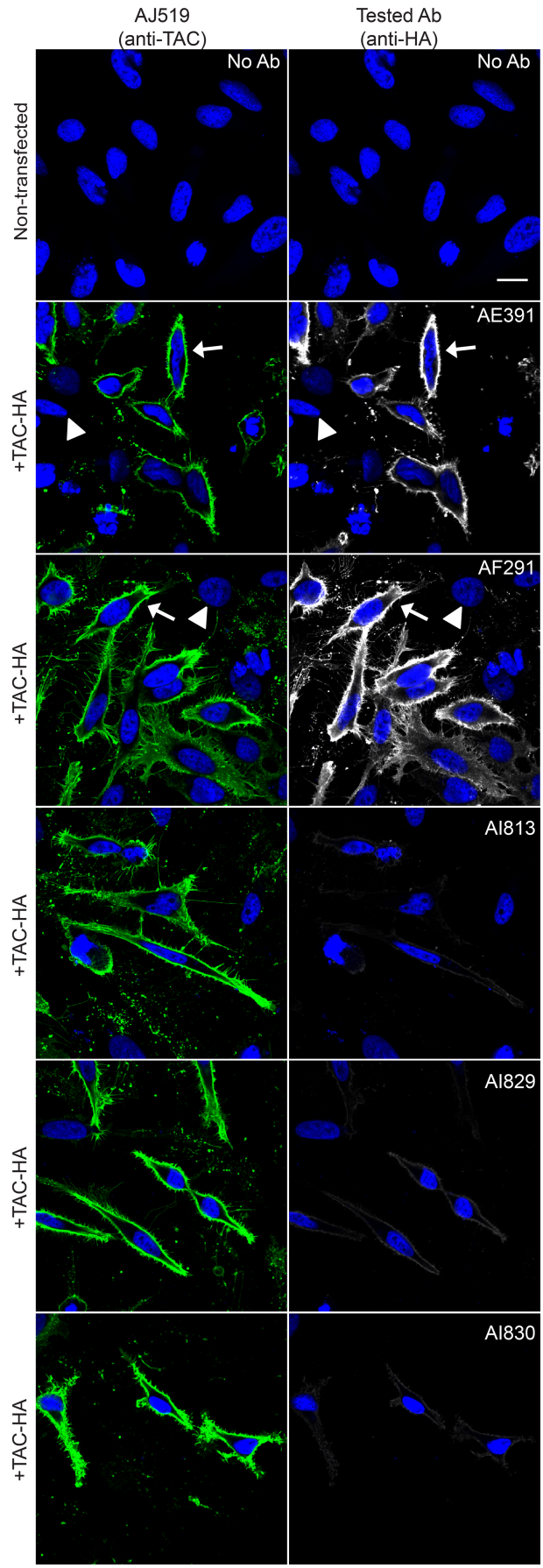

Fig. 1. AE391 and AF291 labeled the plasma membrane of HeLa cells expressing the HA-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the anti-TAC AJ519 antibody (in green); in blue, nuclei were stained with DAPI. No labelling was seen when the primary antibody was omitted, or in non-transfected cells (arrowheads). Scale bar: 20 μm.