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Reference


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The AI196 antibody recognizes a SPOT-tagged recombinant protein by immunofluorescence

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Abstract
The AI196 antibody against the SPOT tag recognizes a SPOT-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells.

Introduction
The SPOT tag (also called BC2 tag) is a short linear epitope derived from the human β-catenin protein (Uniprot #P35222), used for detection and purification of tagged proteins with the BC2-Nb antibody (Braun at al., 2016). Here, we show that the AI196 recombinant antibody, derived from the BC2 nanobody, detects a SPOT-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods
Antibodies: The ABCD_AI196 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima et al., 2020) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies) as a mini-antibody with the antigen-binding VHH fused to a mouse IgG2A Fc. The synthesized VH sequence (GeneArt, Invitrogen) corresponds to the sequences of the variable region of the synthetic camelid antibody BC2-Nb (Rothbauer et al., 2017). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (20 mg/L) was 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 a SPOT-tagged TAC protein (Uniprot #P01589), were used to detect the peptide tag. The SPOT epitope sequence used was PDRVRAVSHWSS 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 SPOT-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 AI196 (final concentration 5 mg/L) and AJ519 antibodies (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, mounted on slides (Menzel-GLäser, 76x26 mm) with Môwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluor oil immersion objective.

Results
The AI196 antibody specifically detected a signal at the plasma membrane in cells transfected with the SPOT-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-SPOT stainings in few non-transfected cells (Fig. 1, arrowheads). No staining was observed when the primary antibody was omitted (Fig. 1, No Ab).

Conflict of interest
The authors declare no conflict of interest.
References


Fig. 1. AL196 labeled the plasma membrane of HeLa cells expressing the SPOT-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.