The AJ517 antibody detects the mouse CD8β protein by flow cytometry

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Abstract

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Introduction

CD8 is a membrane-bound glycoprotein complex expressed primarily in cytotoxic T lymphocytes. It is composed of two transmembrane subunits, α and β, that associate to form a disulfide-linked heterodimer (Parnes, 1989) present at the cell surface. Here, we describe the ability of the AJ517 antibody, a single chain fragment (scFv) derived from the 35.17.2 hybridoma, to successfully detect the CD8β protein (Uniprot #P10300) by flow cytometry in HEK293 cells expressing CD8α and CD8β.

Materials & Methods

Antibodies: ABCD_AJ517 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) was produced by the Geneva Antibody Facility (www.unige.ch/antibodies/) as mini-antibody with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequence (GeneArt, Invitrogen) correspond to the sequence of the variable regions of the 35.17.2 hybridoma (Pierres *et al.*, 1982) joined by a peptide linker $(GGGGS)_3$. The sequencing of the 35.17.2 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. AJ517 supernatant (30 mg/L) was collected after 4 days.

Antigen: The 35.17.2 hybridoma was originally raised against murine leukocytes in Lou/WSl rats (Pierres *et al.*, 1982). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected 3 days before the experiment with the vectors coding for the full-length mouse CD8α (Uniprot #P01731) and CD8β protein. Co-transfection with the CD8α encoding plasmid was performed to guarantee proper protein dimerization and trafficking.

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 the primary antibody AJ517 (3 µg/ml). After two washes in washing buffer, cells were incubated for 20 minutes with secondary goat anti-rabbit IgG conjugated to Alexa Fluor 488 (dilution 1:400, Molecular Probes #A11034). 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

The antibody AJ517 specifically detects the CD8β protein at the cell surface of transfected HEK293 cells expressing CD8α and CD8β. No signal was detected in mock transfected cells (Fig. 1).

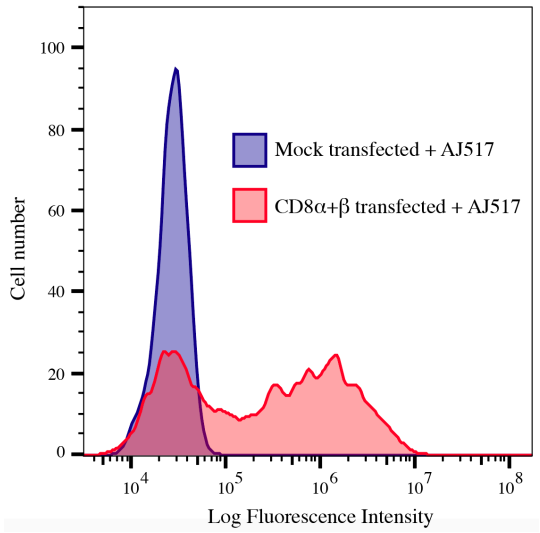

Fig. 1. The AJ517 antibody specifically binds at the cell surface of cells expressing CD8α and CD8β (red). No signal was detected in mock transfected cells incubated (blue).

References

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Conflict of interest

The authors declare no conflict of interest.