The AJ517 antibody labels the mouse CD8β protein by immunofluorescence

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Abstract

The recombinant antibody AJ517 detects by immunofluorescence the mouse CD8β surface protein in paraformaldehyde-fixed cells.

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 immunofluorescence 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 room temperature. Transfected HEK cells were 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 5 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the antibody-containing supernatants (dilution 1:2). After 3 washes (5 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11034). After 3 washes (5 min) with PBS-BSA, cells were 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

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

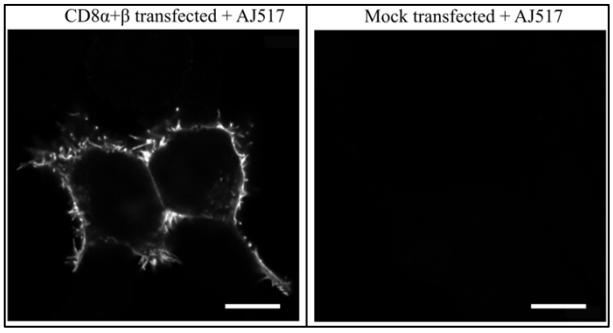

Fig. 1. The AJ517 antibody labels the CD8β cell surface protein in of cells expressing CD8α and CD8β. No staining was observed in mock transfected cells. Scale bar: 10 μm.

References

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Conflict of interest

The authors declare no conflict of interest.