AC650, AC653, AC656 and AD460 antibodies recognize the mouse CD8α protein by flow cytometry

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Abstract
The recombinant antibodies AC650, AC653, AC656 and AD460 detect the mouse CD8α protein by flow cytometry.

Introduction
CD8 protein, composed of two subunits (α and β), is a transmembrane glycoprotein complex expressed primarily in cytotoxic T lymphocytes (Parnes, 1989). Here, we describe the ability of four recombinant antibodies (AC650, AC653, AC656 and AD460) to successfully detect the CD8α protein (Uniprot P01731) in CD8α-transfected HEK293 cells. Two other tested antibodies (AD461 and AJ518) did not.

Materials & Methods
Antibodies: ABCD_AC650, ABCD_AC653, ABCD_AC656, ABCD_AD460, ABCD_AD461 and ABCD_AJ518 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (see Table 1 for clone names and references). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Table 1: Clone number, reference and production yields for the antibodies used in this study.

ABCD	Clone	Reference	Yield (mg/L)
AC650	F03	Schofield *et al*., 2007	100
AC653	E10		60
AC656	B06		70
AD460	YTS 105.18	Shore *et al*., 2006	<5
AD461	OKT8	Kung and Goldstein, 1982	<5
AJ518	19.178	Hennecke and Cosson, 1993	10

Antigen: HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected 2 days before the experiment with a vector coding for the full-length mouse CD8α protein. Cells transfected with an irrelevant plasmid (mock) were used as a negative control.

Protocol: The whole procedure was carried out at 4°C. $1x10^6$ 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 recombinant antibodies (5 mg/L in PBS-BSA). After two washes in washing buffer, cells were incubated for 20 minutes with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (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 (Beckman Coulter CytoFLEX).

Results
Antibodies AC650, AC653, AC656 and AD460 detected the CD8α protein in CD8α-transfected HEK293 cells. No signal was detected in mock-transfected cells (Fig. 1). AD461 and AJ518 did not recognize the CD8α protein by flow cytometry. For AD461, this might be due to the fact that this antibody is poorly produced. All these antibodies were also tested at lower concentration (1 mg/L) in PBS-BSA. Only antibodies AC653, AC656 and AD460 exhibited a positive signal at this concentration (data not shown).

References

Hennecke S, Cosson P. Role of transmembrane domains in assembly and intracellular transport of the CD8 molecule. J Biol Chem. 1993; 268(35):26607-12. PMID: 8253791.

Kung P, Goldstein G. Complement-fixing monoclonal antibody to human suppressor T cells and methods of preparing same. United States, US4361550, 1982.

Parnes JR. Molecular biology and function of CD4 and CD8. Adv Immunol 1989; 44:265-311. PMID: 2493728.

Schofield DJ, Pope AR, Clementel V, *et al*. Application of phage display to high throughput antibody generation and characterization. Genome Biol. 2007; 8(11):R254. PMID: 18047641.

Shore DA, Teyton L, Dwek RA, Rudd PM, Wilson IA. Crystal structure of the TCR co-receptor CD8α in complex with monoclonal antibody YTS 105.18 Fab fragment at 2.88Å resolution. J Mol Biol. 2006; 358(2):347-54. PMID: 16530222.

Conflict of interest

The authors declare no conflict of interest.

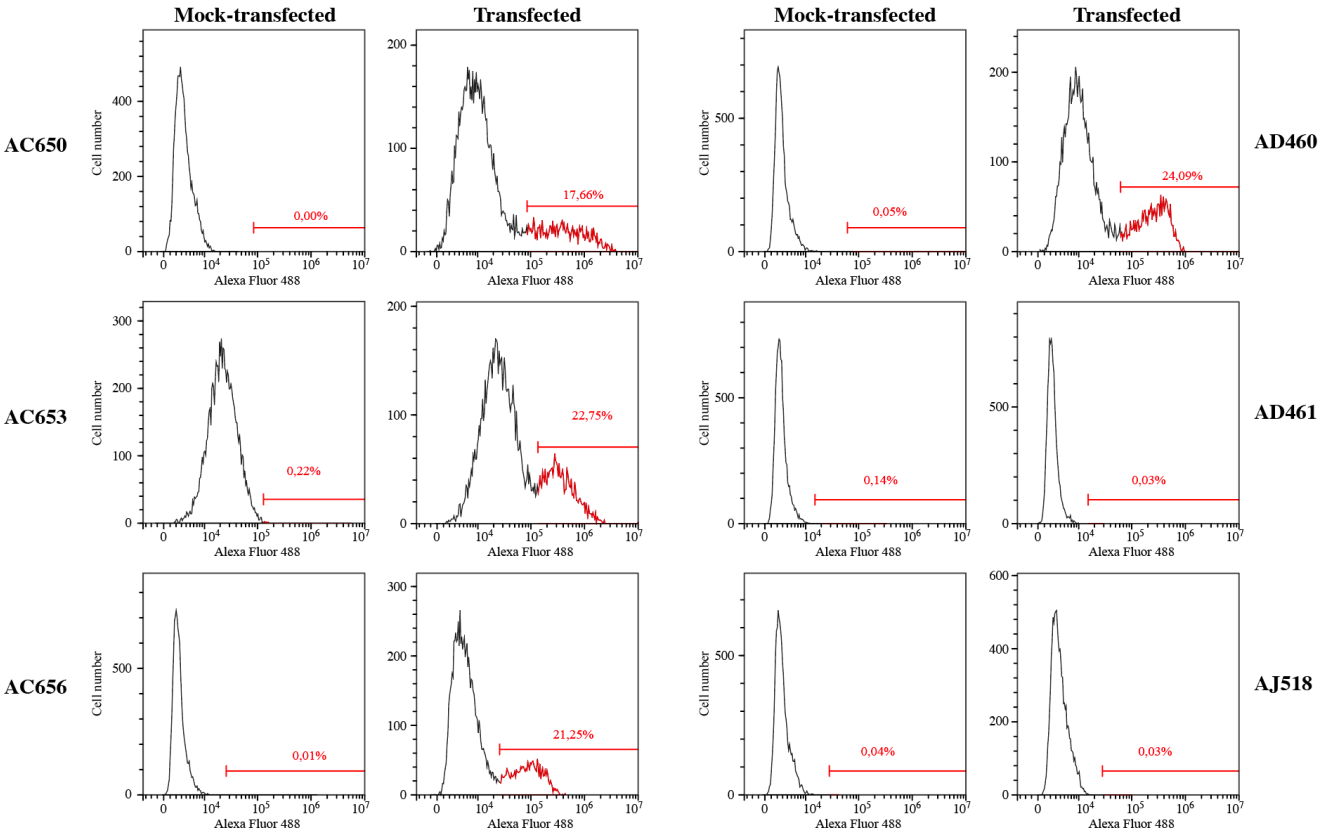

Fig. 1. Mono-parametric representation of flow cytometry analysis depicting the Alexa Fluor 488 signal. AC650, AC653, AC656 and AD460 antibodies labeled HEK293 transfected cells overexpressing the CD8α protein. No signal was detected in mock-transfected cells. AD461 and AJ518 did not recognize the CD8α protein by flow cytometry. Transfection efficiency was estimated to be around 20%.