AC650, AC653, AC656 and AD460 antibodies label the mouse CD8α protein by immunofluorescence

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

Introduction
The CD8 glycoprotein is composed of two transmembrane subunits, α and β. Its expression, on the surface of cytotoxic T lymphocytes, contributes to drive the specific interaction between the T-cell receptor and class-I major histocompatibility complex molecules on target cells (Wong and Pamer, 2003). Here, we describe the ability of four recombinant antibodies (AC650, AC653, AC656 and AD460) to successfully detect the mouse CD8α protein (Uniprot P01731) by immunofluorescence 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 cells (growing in DMEM, Gibco 11960044, supplemented with 10% FBS) were transiently transfected 2 days before the experiment with a vector coding for the full-length mouse CD8α. Cells transfected with an irrelevant plasmid (mock-transfected) were used as a negative control.

Protocol:
The whole procedure was carried out at room temperature. Transfected HEK293 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 recombinant antibodies (5 mg/L in PBS-BSA). 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
Antibodies AC650, AC653, AC656 and AD460 recognized the mouse CD8α protein addressed at the cell surface of CD8α transfected cells (Fig. 1). The weak signal observed with the antibody AD460 is probably due to the fact that this antibody is poorly produced. The absence of staining in mock-transfected cells indicates the specificity of the signal observed (Fig. 1). AD461 and AJ518 did not recognize the CD8α protein; for AD461, this might be due to the fact that this antibody is poorly produced.

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.

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.

Wong P, Pamer EG. CD8 T cell responses to infectious pathogens. Annu Rev Immunol. 2003; 21:29-70. PMID: 12414723.

Conflict of interest

The authors declare no conflict of interest.

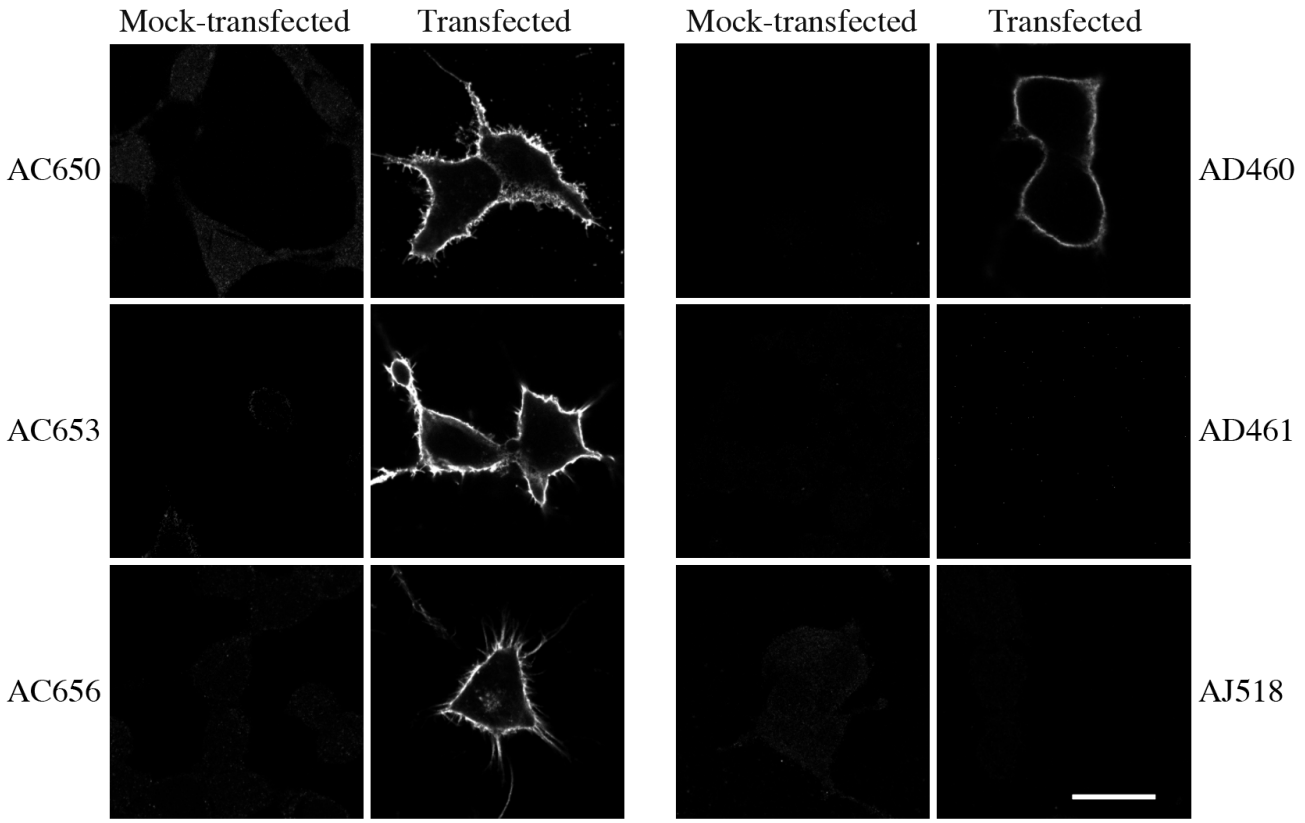

Fig. 1. AC650, AC653, AC656 and AD460 specifically labeled transfected HEK293 cells expressing the mouse CD8α protein. AD461 and AJ518 did not recognize the CD8α protein. No labeling was seen in mock-transfected cells. Scale bar: 20 μm.