AD822, AW199, AW200 and AW201 antibodies against the human Fas receptor (CD95) bind the surface of HEK293 cells as revealed by flow cytometry

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Abstract
The recombinant antibodies AD822, AW199, AW200 and AW201 bind HEK293 cells expressing the human Fas receptor protein as assessed by flow cytometry.

Introduction
The Fas receptor (or CD95, UniProt #P25445) is a transmembrane protein expressed at the cell surface. Binding of the Fas receptor to its ligand FasL leads to cell apoptosis (Itoh *et al*., 1991). Here, we tested by flow cytometry the ability of 6 recombinant antibodies (AD822, AW199, AW200 and AW201, AD825 and AJ757) to bind HEK293 cells that are known to express the Fas receptor (Uhlèn *et al*., 2015).

Materials & Methods
Antibodies: ABCD_AD822, ABCD_AD825, ABCD_AW199, ABCD_ AW200, ABCD_AW201, and ABCD_AJ757 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. (see Table 1 for clone names and references). HEK293 suspension cells (growing in HEK TF medium, Xell #861-0001, supplemented with 0.1% Pluronic F68, Sigma #P1300) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants were collected after 4 days (see Table 1 for individual yields).

ABCD	*Clone*	*Reference*	*Yield (mg/L)*
AD822	h-HFE7A	Haruyama *et al*., 2002	10
AD825	huF919	Kamon *et al*., 2000	85
AW199	E09	Chodorge *et al*., 2012	90
AW200	E01		80
AW201	E03		80
AJ757	APO-1	Nalivaiko *et al*., 2016	<5

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

Protocol: The whole procedure was carried out at 4°C. $1x10^6$ HEK293 cells were pelleted and washed once in washing buffer (PBS + 0.2% BSA (w/v)). Cells were then incubated for 20 minutes with the recombinant antibodies (5 mg/L in 500 µL of PBS-BSA, excepted for the antibody AJ757 that was used undiluted). After two washes in 1 mL of washing buffer, cells were incubated for 20 minutes with 500 µL of secondary goat anti-rabbit IgG antibody conjugated to AlexaFluor-488 (1:400, Molecular Probes#A11034). After four washes in 1 mL of washing buffer, cells were resuspended in 500 µL of washing buffer and analyzed with a CytoFLEX S4 flow cytometer (Beckman Coulter). Dead cells were excluded after staining with Dapi (5 mg/L) and gating on Dapi negative cells.

Results
Antibodies AD822, AW199, AW200 and AW201 were able to bind to the HEK293 cells most probably via the Fas receptor protein present at the surface. No signal was detected when the primary antibody was omitted (Fig. 1). In our experimental conditions, AD825 and AJ757 did not label HEK293 cells and thus presumably did not recognize the Fas receptor protein. For AJ757, this might be due to the fact that this antibody is poorly produced. The same experiments performed using a lower antibody concentration (1 mg/L) yielded similar results (data not shown). Further experiments (ideally using specific KO cells) will be necessary to confirm the specific binding of AD822, AW199, AW200 and AW201 to the Fas receptor protein at the surface of the HEK293 cells suggested in this study.

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Conflict of interest

Tania Jauslin is an associate-editor of the journal Antibody Reports.

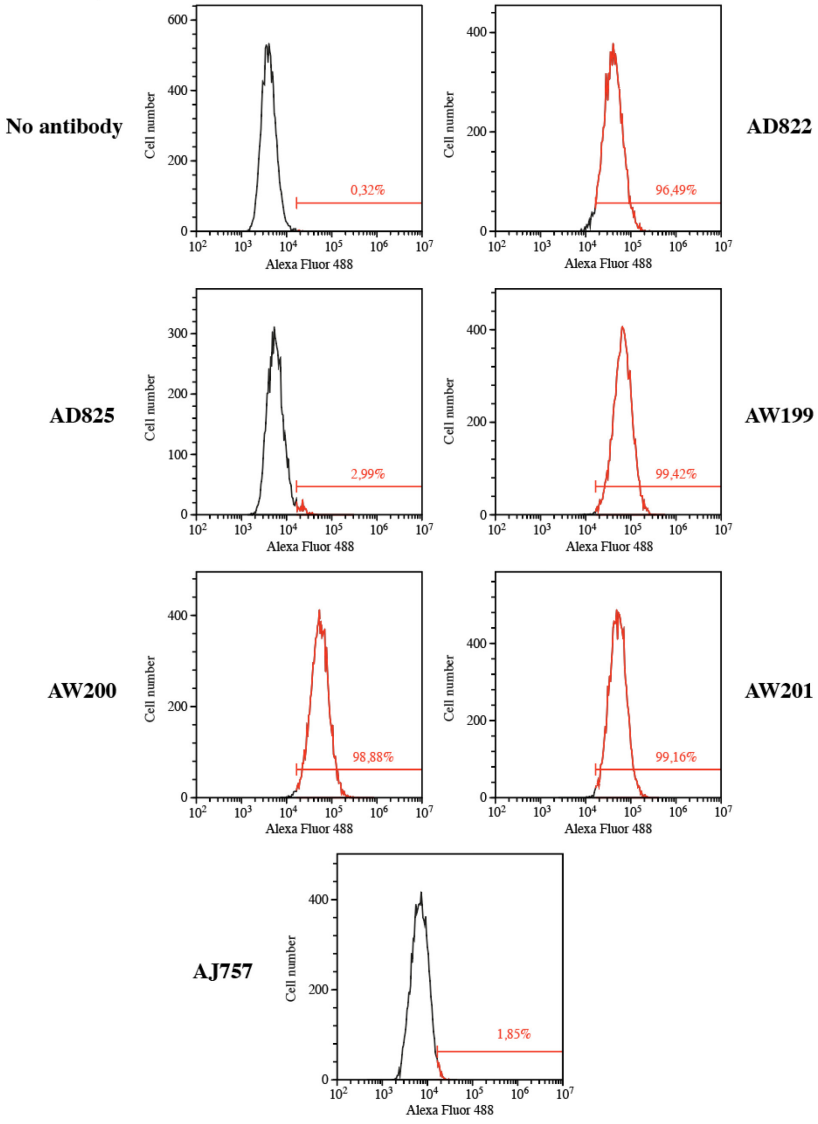

Fig. 1. Mono-parametric representation of flow cytometry analysis depicting the Alexa Fluor 488 signal. AD822, AW199, AW200 and AW201 antibodies labeled the Fas receptor protein in HEK293 cells. No signal was detected when primary antibody was omitted. AD825 and AJ757 did not recognize the Fas receptor by flow cytometry.