AF641, AI954 and AW954 antibodies label the endoplasmic reticulum by immunofluorescence

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Abstract
The AF641, AI954 and AW954 recombinant antibodies detect different markers of the endoplasmic reticulum by immunofluorescence in HEK293 cells.

Introduction
BiP (binding immunoglobulin protein, also known as GRP78) and endoplasmin are molecular chaperones localized in the endoplasmic reticulum (ER). These proteins play critical roles in the folding of proteins in the secretory pathway (Melnick *et al*., 1994; Csermely *et al*., 1995). A KDEL sequence at the luminal C-terminal end of BiP, endoplasmin, and other proteins ensures their specific localization in the ER (Munro and Pelham., 1987). Here, we tested the ability of six recombinant antibodies to detect different markers of the ER by immunofluorescence in YFP-KDEL transfected HEK293 cells. Among the tested antibodies, AF641, AI954, AF650 and AF637 were reported to detect the human BiP protein (Uniprot#P11021). The KDEL motif was recognized by the antibody AW954 and the human endoplasmin (Uniprot #P14625) by AP498.

Materials & Methods
Antibodies: ABCD_AF637, ABCD_AF641, ABCD_AF650, ABCD_AI954, ABCD_AP498 and ABCD_AW954 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 a vector coding for the scFv-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

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 yellow fluorescent protein (YFP) fused to the KDEL motif at the carboxy-terminal position.

ABCD	*Clone*	*Reference*	*Yield (mg/L)*	*Target*
AF637	GC-20	Naoki *et al*., 2010	<5	BiP
AF641	B4	Pasqualini *et al*., 2018	20	
AF650	2D6F9	Hallahan and Yan, 2017	20	
AI954	MAb159	Gill and Liu, 2018	<5	
AP498	H11B	Arnold-Shild *et al*., 2000	120	Endoplasmin
AW954	VHH 5	Klooster *et al*., 2009	10	KDEL

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

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 + 0.1% Triton X-100 (v/v) for 5 min, washed once (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 20 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-647 (1:400, Thermofisher #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 LSM800 confocal microscope, with a 63x Neofluar oil immersion objective

Results
Since the KDEL motif acts as an ER targeting motif, cells expressing YFP-KDEL were used to visualize the ER compartment (Fig.1). The signal observed with AW954, AF641 and AI954 co-localized with the overexpressed YFP-KDEL protein demonstrating that these antibodies specifically detected the ER compartment. (Fig. 1). In the experimental conditions used in this study, no signal was observed with AF637, AF650 and AP498 antibodies or when the primary antibody was omitted. The absence of signal with AF637 might be due to the fact that this antibody is poorly produced. Note that the use of different experimental conditions (e.g. a different fixation procedure) may modify strongly the reactivity of antibodies.

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Conflict of interest

The authors declare no conflict of interest.

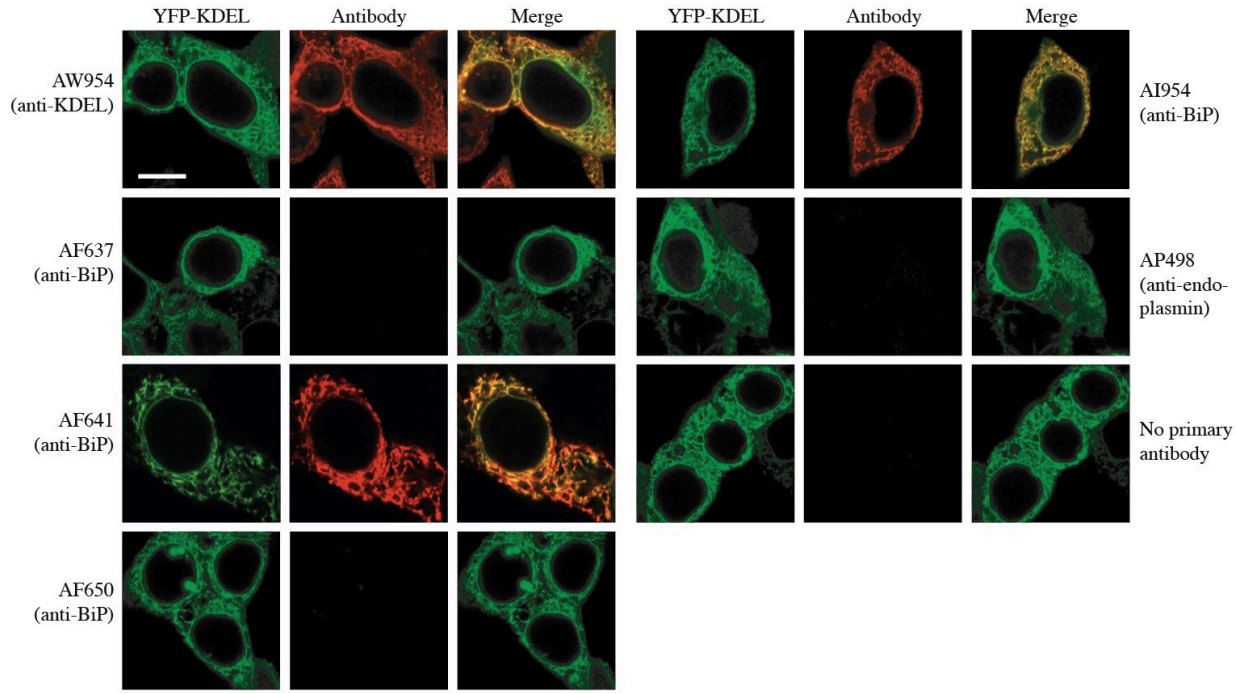

Fig. 1. AW954, AF641 and AI954 specifically label the endoplasmic reticulum in transfected HEK293 cells expressing the YFP-KDEL protein. No signal was observed with AF637, AF650 and AP498 antibodies, or when the primary antibody was omitted. Scale bar: 10 µm.