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Article

2023

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Mencier, Lucie Louise; Moudarres, Lana; Najand, Gaëlle Aude; Roch, Laura; Rüttsche, Audrey; Schwitzguebel, Valérie; Seddiki, Wissem; Soulie, Paola Maria Flavia Noëlle; Anagbogu, Nneka Mukaosulu; Bennani, Kenza; Brun, Elise Joséphine; Butterworth, Hannah Louise; Carry, Jennifer; Dawit Gebreyohannes, Soyan [and 12 more]

How to cite

MENCIER, Lucie Louise et al. AF641, AI954 and AW954 antibodies label markers of the human endoplasmic reticulum by western blot. In: Antibody reports, 2023, vol. 6, n° 1, p. e1227. doi: 10.24450/journals/abrep.2023.e1227

This publication URL: <https://archive-ouverte.unige.ch/unige:170574>

Publication DOI: [10.24450/journals/abrep.2023.e1227](https://doi.org/10.24450/journals/abrep.2023.e1227)

AF641, AI954 and AW954 antibodies label markers of the human endoplasmic reticulum by western blot

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Abstract

The recombinant antibodies AF641, AI954 and AW954 detect different markers of the endoplasmic reticulum by western blot.

Introduction

BiP (UniProt #P11021; binding immunoglobulin protein, also known as GRP78) and endoplasmin (UniProt #P14625) are molecular chaperones localized in the endoplasmic reticulum (ER). BiP and endoplasmin 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). In the present study, we assessed the ability of six recombinant antibodies to detect BiP, endoplasmin and the KDEL motif in HEK293 cells by western blot. Antibodies AF641 and AI954 were able to recognize the human BiP protein. The KDEL motif was detected by the antibody AW954. The other tested antibodies failed to label either BiP (AF650 and AF637) or the human endoplasmin (AP498).

Materials & Methods

Antibodies: ABCD_AF637, ABCD_AF641, ABCD_AF650, ABCD_AI954, ABCD_AP498 and ABCD_AW954 antibodies (ABCD nomenclature, <http://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (<http://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.

ABCD	Clone	Reference	Yield (mg/L)	Target
AF637	GC-20	Naoki <i>et al.</i> , 2010	<5	BiP
AF641	B4	Pasqualini <i>et al.</i> , 2018	20	
AF650	2D6F9	Hallahan and Yan, 2017	20	
AI954	MAB159	Gill and Liu, 2018	<5	
AP498	H11B	Arnold-Shild <i>et al.</i> , 2000	120	Endoplasmin
AW954	VHH 5	Klooster <i>et al.</i> , 2009	10	KDEL

Table 1: Clone numbers, references, production yields and targets of the antibodies used in this study.

Protocol: 2.5×10^5 HEK293 cells were pelleted, washed once in PBS and lysed in PBS containing 0.5% (v/v) Triton X-100. Nuclei were pelleted by centrifugation (5 min at 12'000 g) and the supernatant was recovered and mixed with reducing or non-reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, +/- 6% (v/v) β -mercaptoethanol). Each sample was separated by electrophoresis on an acrylamide gel 4-20% (SurePAGE Bis-Tris, Genscript #M00655) (200 V, 30 min), and transferred onto a nitrocellulose membrane using a dry transfer system for 10 min (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked 2 h in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk and washed three times for 5 min in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with the recombinant antibodies (5 mg/L in PBS-Tween-milk) for 20 min at room temperature and washed three times for 5 min. The membranes were then incubated for 20 min with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich #A8275, dilution 1:3000 in PBS-Tween-milk) and washed 5 times for 5 min in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging system (Syngene).

Results

Both antibodies AF641 and AI954 recognize a similar single band in reducing and non-reducing conditions at approximately 80 kDa. The observed signal is slightly higher than the expected theoretical size of the human BiP protein (72 kDa) and may be due to known post-translational modifications of the BiP protein. No signal was detected with others anti-BiP antibodies (AF637 and AF650, Fig. 1). The absence of signal with AF637 might be due to the fact that this antibody is poorly produced. The antibody AW954 recognizes several proteins which are presumed to bear a C-terminal KDEL retention motif (Fig. 1). One of the proteins detected by AW954 migrates with an apparent size of 80 kDa and may correspond to the BiP protein.

No signal was observed with the AP498 anti-endoplasmin antibody.

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Conflict of interest

The authors declare no conflict of interest.

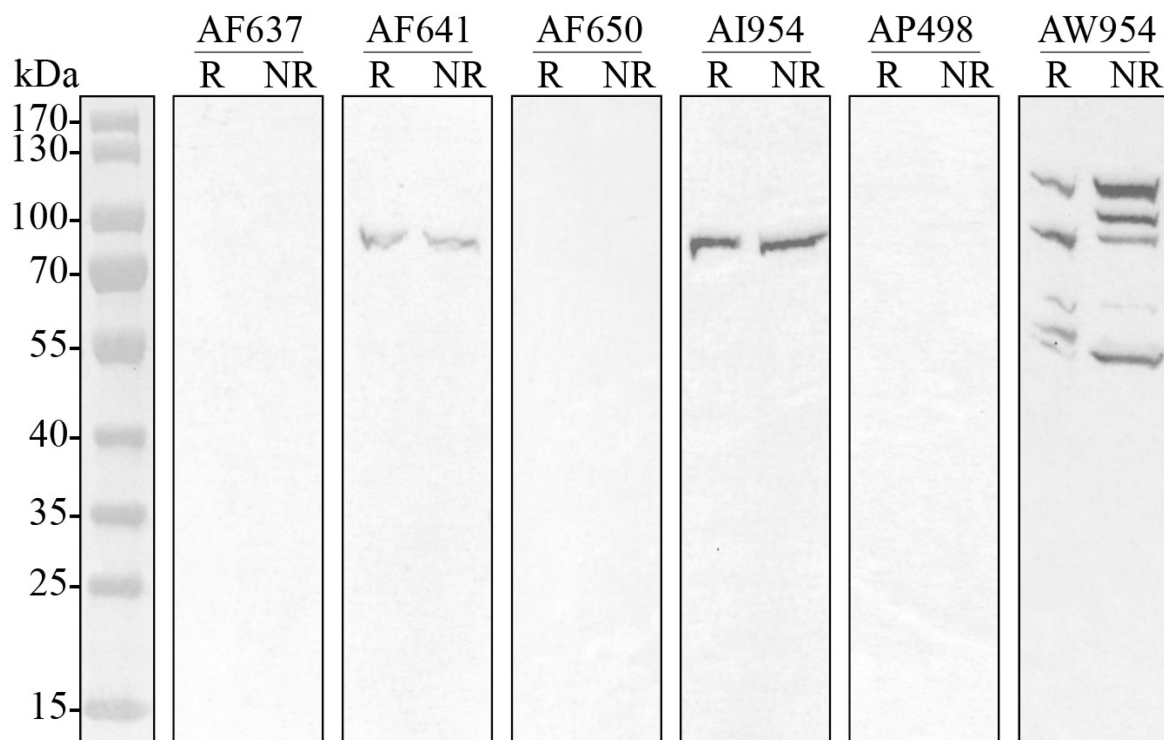


Fig. 1. AF641, AI954 and AW954 specifically label markers of endoplasmic reticulum in HEK293 cells in both reducing (R) and non-reducing (NR) conditions. No signal was observed with AF637, AF650 and AP498 antibodies.