The AW288 and AX067 antibodies recognize human actin by immunofluorescence in HEK cells

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Abstract

The AW288 and AX067 antibodies recognize human actin in paraformaldehyde-fixed HEK cells by immunofluorescence.

Introduction

Actin is one of the most abundant proteins in eukaryotic cells, and a major structural component of the cytoskeleton (Pollard, 2016). Two recombinant antibodies (AW288 and AX067) were tested for their ability to label actin by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AW288 and ABCD_AX067 antibodies (https://web.expasy.org/abcd/, ABCD nomenclature) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding domain fused to a rabbit IgG Fc. The synthesized VHH sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of clones NactNB (for AW288, Knoll *et al.*, 2018) and anti-Actin VHH (for AX067, Gil *et al.*, 2020). 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 VHH-Fc. Supernatants (150 and 120 mg/L, respectively) were collected after 4 days.

The antibody AJ519, which recognizes the TAC antigen (human IL2RA, Uniprot P01589), was used as a negative control (Arsimoles *et al.*, 2020).

Antigen: HEK cells were cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and grown in DMEM GlutaMAX™ (Gibco 31966) supplemented with 8% Fetal Bovine Serum (Gibco 10270).

Protocol: The whole procedure was carried out at room temperature. Cells were rinsed once with PBS, and fixed either with *(i)* PBS + 4% paraformaldehyde (PAF) (w/v) (Applichem A3013) for 30 min, blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem A3661) for 5 min, and then permeabilized in PBS + 0.2% saponin for 5 min; or *(ii)* methanol at -20 ºC for 2 min. Fixed cells were washed once (5 min) in PBS and once with PBS + 0.2% (w/v) BSA (PBS-BSA), and incubated for 30 min with the primary antibodies (final concentration 5 mg/L in PBS-Tween). After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-rabbit IgG conjugated to AlexaFluor-647 (1:400, Molecular Probes A21245). After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min with Phalloidin-TRITC (1 µg/ml in PBS-BSA, Sigma P1951). After 3 washes (10 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

TRITC-labelled phalloidin was used as a marker to visualize polymerized actin filaments in PAF-fixed HEK cells (Fig. 1, white). Two anti-actin antibodies, AW288 and AX067, label some of the same structures stained with phalloidin (Fig. 1, cyan), identified by arrows in Fig. 1B. AW288 and AX067 also label the cellular cytoplasm (Fig. 1B, pinheads). As discussed previously (Marchetti, 2022a), this cytosolic staining might represent non-polymerized actin.

No staining was observed when the primary irrelevant AJ159 antibody was used as a negative control (Fig. 1, 'Neg ctr'). AW288 and AX067 did not label methanol-fixed cells (data not shown).

AW288 and AX067 were also tested for detection of actin by western blot (protocol exactly as described in Marchetti, 2022b). Neither antibody detected any specific bands (data not shown).

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Conflict of interest

The authors declare no conflict of interest.

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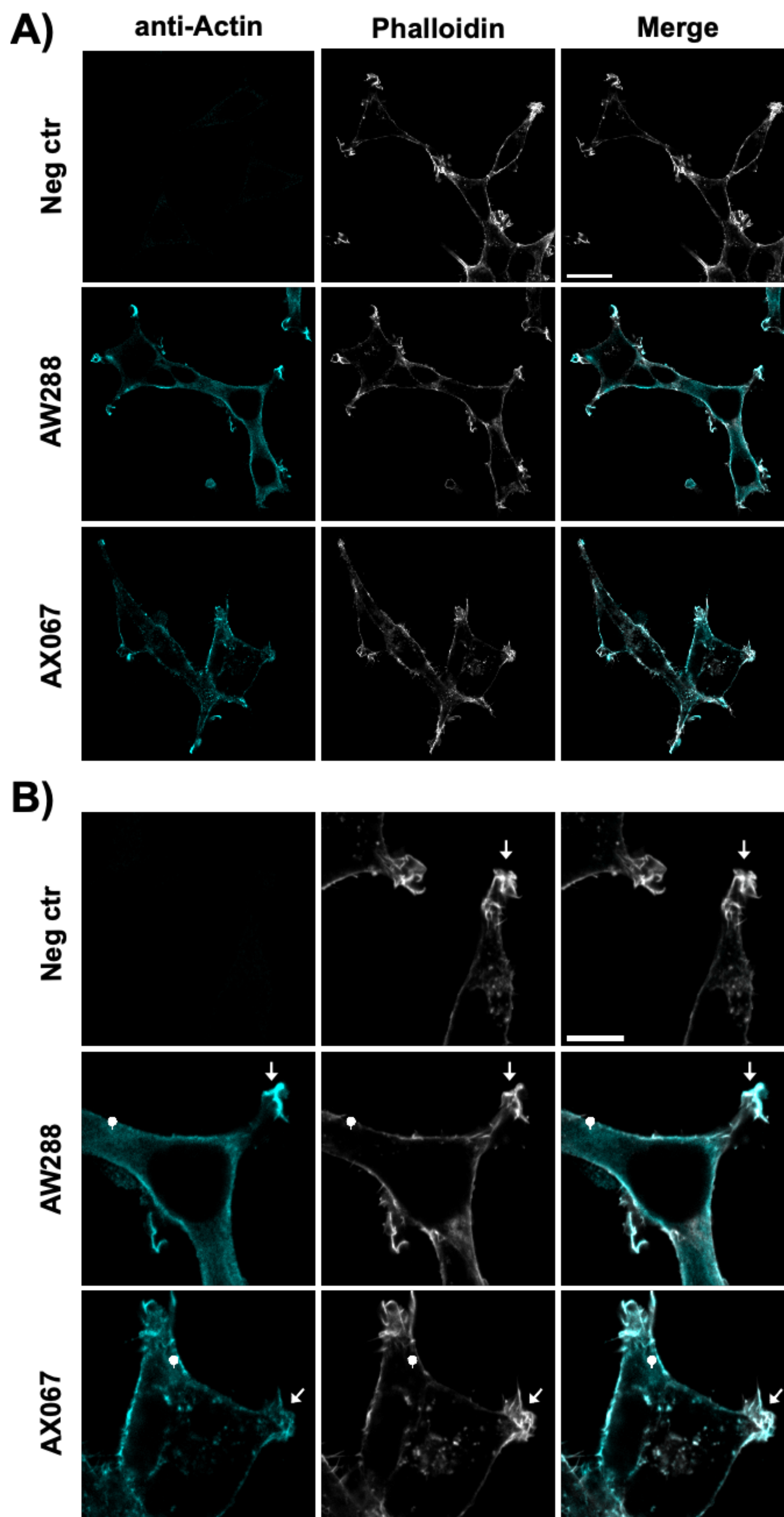

Fig. 1. Actin labelling in PAF-fixed HEK cells. (A) Labeling with AW288 and AX067 antibodies ('anti-Actin', in cyan) partially co-localizes with phalloidin staining ('Phalloidin', in white). Labeling of cytoplasmic structures not labelled by phalloidin was also seen. No labelling was observed when an unrelated primary antibody (anti-TAC AJ519) was used ('Neg ctr' panel). Scale bar: 20 μm. **(B)** Insets from images shown in (A), showing partial co-localization of AW288 and AX067 with phalloidin (arrows) and the labelling of cytoplasmic structures not labelled by phalloidin (pinheads). Scale bar: 10 μm.

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