The AE765, AK423, AK692, AO233 and AO234 antibodies recognize human actin by immunofluorescence in HEK cells

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Abstract

The AE765, AK692, AO233 and AO234 antibodies recognize human actin in PAF-fixed HEK cells by immunofluorescence. Phalloidin, which recognizes polymerized actin, is more adequate to label actin filamentous structures. The AK423 antibody recognizes human actin in methanol-fixed HEK cells, but in these conditions the actin network is poorly preserved.

Introduction

Actin is one of the most abundant proteins in eukaryotic cells, and a major structural component of the cytoskeleton, forming networks of microfilaments in the cytoplasm of cells. The human genome contains six genes for actin (three for α-actin, one for β-actin, and two for γ-actin) (Pollard, 2016). Five recombinant antibodies (AE765, AK423, AK692, AO233 and AO234) were tested for their ability to label actin by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AE765, ABCD_AK423, ABCD_AK692, ABCD_AO233 and ABCD_AO234 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 scFv fused to a rabbit IgG Fc. The synthesized scFv or VHH sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (Table 1). 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 or VHH-Fc. Supernatants (see Table 1 for individual yields) 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). As positive control, a commercial anti-beta actin antibody (clone 2D4H5, Proteintech 66009-1-Ig), raised against human ACTB (Uniprot P60709), was used.

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

ABCD	Clone	Epitope	Reference	Yield (mg/L)
AE765	SA1A	Human actin and alpha-actinin	Victor *et al.*, 1992	70
AK423	mAb 236	*Dictyostelium* actin (Uniprot P07830)	Lima, 2019	10
AK692	Nb141	Human ACTB, (Uniprot P60709)	Jovčevska *et al.*, 2014	<5
AO233	3-1		Persson *et al.*, 2013	90
AO234	3-2			30

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 °C 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 (for the ABCD antibodies, final concentration 5 mg/L in PBS-Tween; for the Proteintech antibody, 0.05 mg/L). 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, for the ABCD antibodies) or anti-mouse IgG conjugated to AlexaFluor-647 (1:400, Molecular Probes A21235, for the Proteintech antibody). 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

Fluorescent phalloidin was used as a marker to label polymerized actin filaments in PAF-fixed HEK cells (Fig. 1, in white). Four anti-actin antibodies, AE765, AK692, AO233 and AO234, label some of the same structures stained with phalloidin (Fig. 1, in cyan; zoomed-in regions, identified by arrows, can be seen in Fig. 2). They also label the cellular cytoplasm (Fig. 2, pinheads). Although this cytosolic staining may represent a background staining, it seems more likely that it is due to detection of non-polymerized actin.

No staining was observed when the primary anti-TAC antibody was used as a negative control (Fig. 1, 'Neg ctr'). In PAF-fixed HEK cells, no staining was observed with the commercial anti-actin 2D4H5 antibody, nor with AK423 (Figs. 1 and 2).

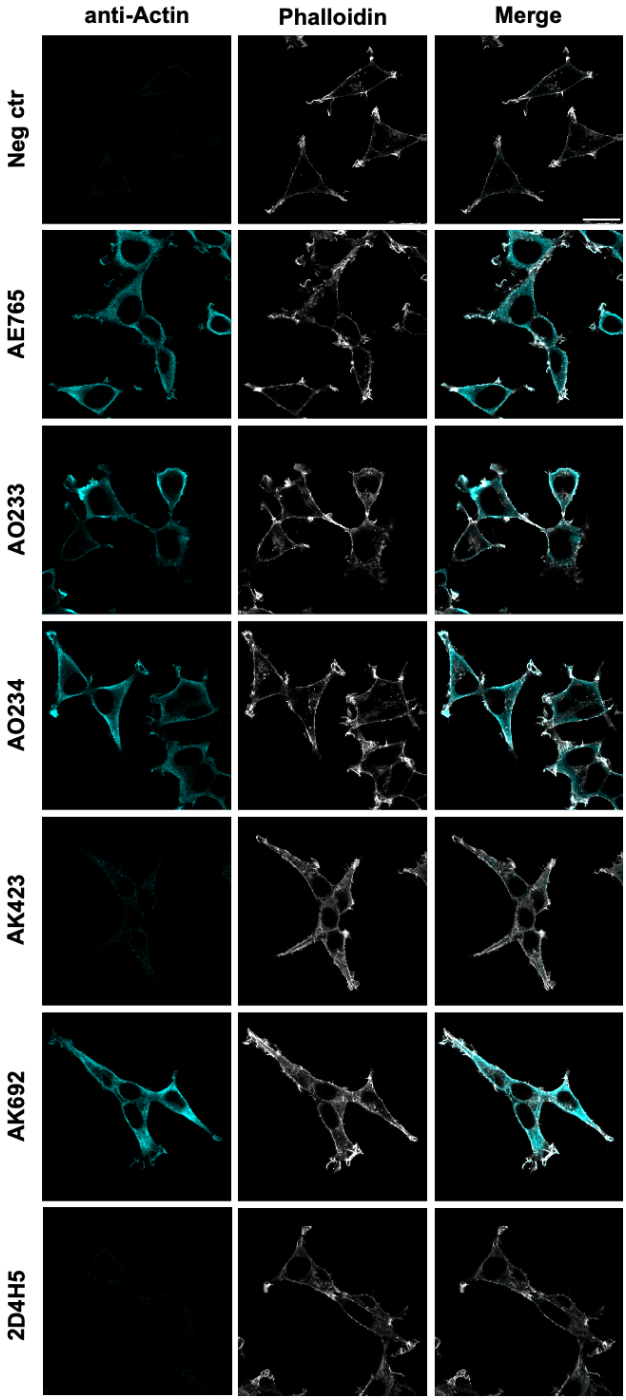

Fig. 1. Actin labelling in PAF-fixed HEK cells. Labeling with AE765, AK692, AO233 and AO234 antibodies ('anti-Actin', in cyan) partially co-localizes with phalloidin staining ('Phalloidin', in white). Labeling of cytoplasmic structures not labelled by phalloidin was also seen. No labelling was observed when an unrelated primary antibody (anti-TAC AJ519) was used ('Neg ctr' panel). No labelling was seen with AK423 or the commercial 2D4H5 antibodies. Scale bar: 20 µm.

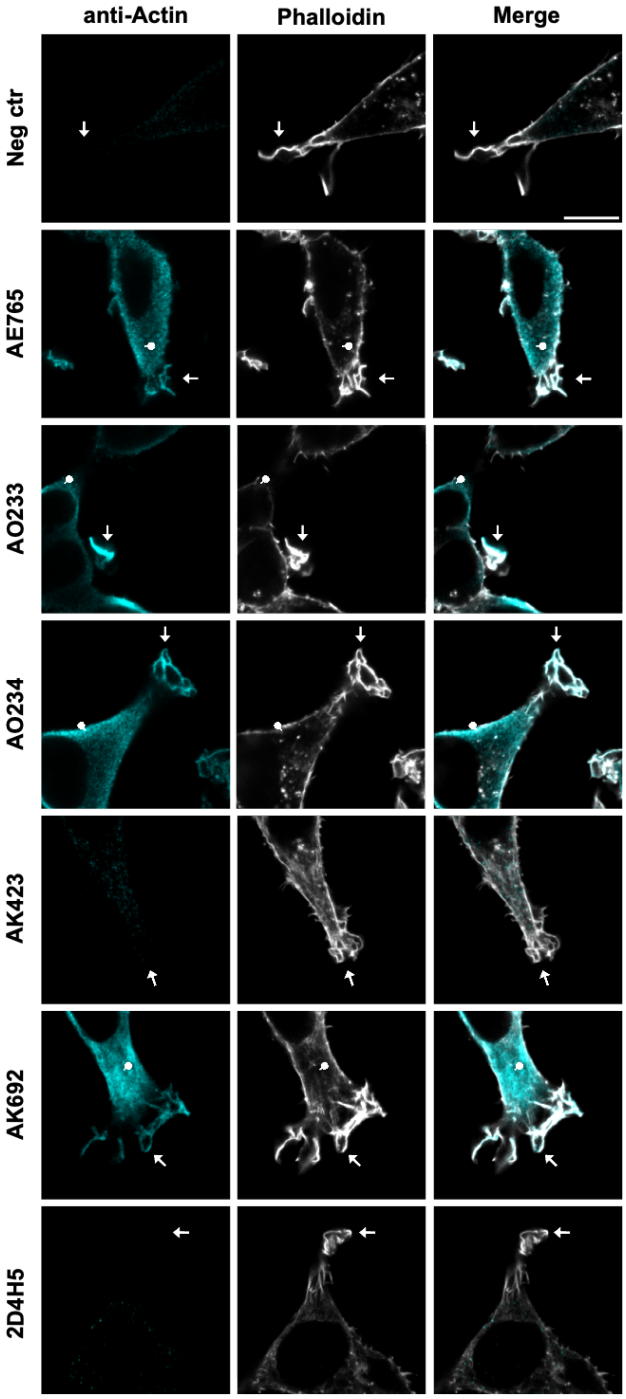

Fig. 2. Actin labelling in PAF-fixed HEK cells. Insets from Fig. 1, showing partial co-localization of AE765, AK692, AO233 and AO234 with phalloidin (arrows) and the labelling of cytoplasmic structures not labelled by phalloidin (pinheads). Scale bar: 10 µm.

Methanol fixation abrogates the signal of phalloidin (as it destroys the native conformation of the F-actin; Mierke, 2018). In methanol-fixed HEK cells, AE765, AK692, AO233 and AO234 did not label any recognizable structures (Fig. 3).

On the contrary, both the commercial anti-actin 2D4H5 and AK423 antibodies label structures that likely represent poorly conserved filamentous actin structures (Fig. 3, arrows).

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Conflict of interest

The authors declare no conflict of interest.

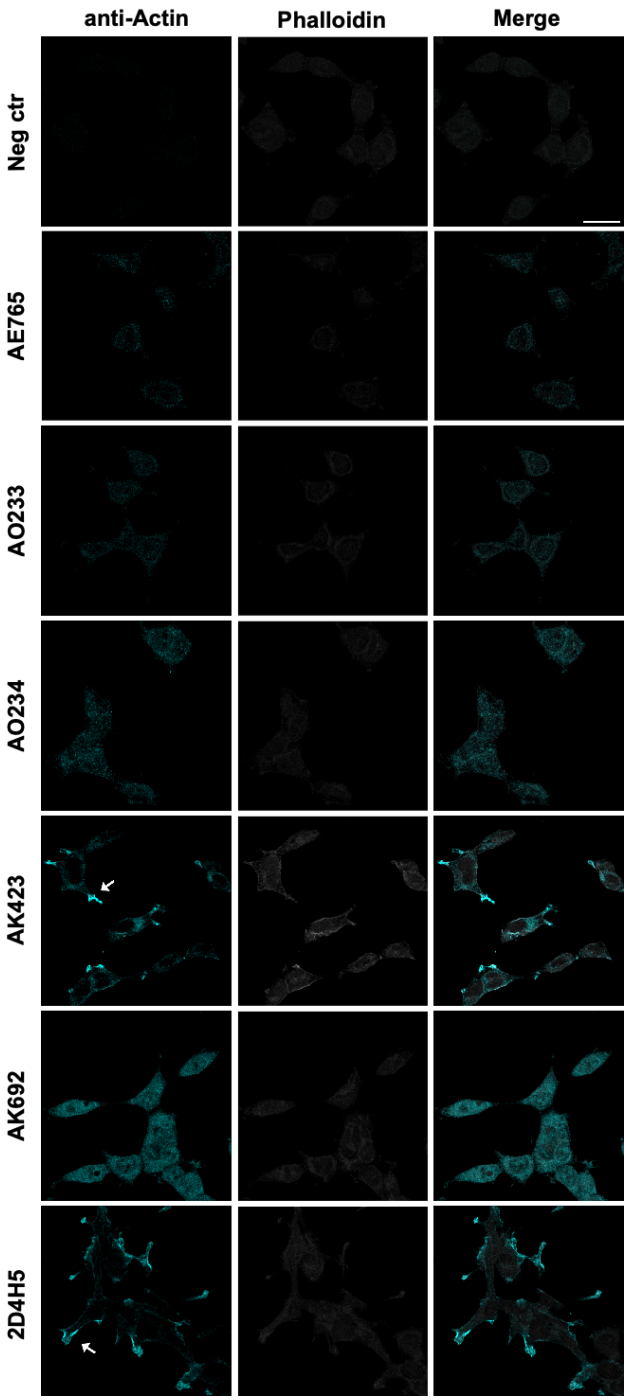

Fig. 3. Actin labelling in methanol-fixed HEK cells. No phalloidin staining was seen in methanol-fixed cells ('Phalloidin', in white). Labeling with AE765, AK692, AO233 and AO234 antibodies ('anti-Actin', in cyan) was also abrogated. No labelling was seen when an unrelated primary antibody (anti-TAC AJ519) was used ('Neg ctr' panel). AK423 and the commercial 2D4H5 antibodies label actin structures (arrows). Scale bar: 20 μm.