The AK423 antibody recognizes human actin by western blot

Anna Marchetti

Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The AK423 antibody, raised against *Dictyostelium* actin, recognizes human actin by western blot; AE765, AK692, AO233 and AO234 do not.

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). The AK423 antibody, originally raised against actin from the amoeba *Dictyostelium discoideum*, also recognizes human actin by western blot; four other recombinant antibodies (AE765, AK692, AO233 and AO234), originally targeting human actin, do not.

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 $(GGGGS)_3$ (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 or VHH-Fc. Supernatants (see Table 1 for individual yields) were collected after 4 days.

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 grown in DMEM GlutaMAX™ (Gibco 31966) supplemented with 8% Fetal Bovine Serum (Gibco 10270) were used.

Protocol: $5x10^6$ cells were pelleted and lysed for 15 min in 100 µL of ice-cold lysis buffer (25 mM Tris-HCl pH 7.4 + 0.5 % Triton X-100 + 120 mM NaCl) containing protease inhibitors. Lysate was centrifuged 15 min, 10'000 g at 4 °C to remove nuclei. One volume of sample buffer was added to the lysate (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, with or without 6% (v/v) β-mercaptoethanol) and boiled for 15 min at 95 °C. Dilutions of each sample (500'000, 100'000, 20'000 and 4'000 cells) were migrated (150 V, 45 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen IB23001). The membranes were blocked during 60 min in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed once for 15 minutes in PBS + 0.1% (v/v) Tween20 (PBS-Tween). The membranes were then incubated overnight at RT with the anti-actin antibodies (for the ABCD antibodies, final concentration 5 mg/L in PBS-Tween; for the Proteintech antibody, 0.05 mg/L). The membranes were then washed three times (15+15+10 min) in PBS-Tween, incubated for 1 hour with the horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich A8275, dilution 1:3000, for the ABCD antibodies) or anti-mouse IgG (Biorad 170-6516, dilution 1:3000, for the Proteintech antibody) and washed three times (15 min) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results

Of the five anti-actin antibodies tested, only AK423 specifically recognized actin from HEK cells, detecting a band around 45-50 kDa, similar to the positive control (Fig. 1). AK423 also cross-reacted with a band of higher molecular weight (~130-170 kDa). The other four antibodies (AE765, AK692, AO233 and AO234) did not detect any specific bands (Fig. 1).

Fig. 1 shows results for reduced samples; non-reduced samples show exactly the same migration and detection pattern (data not shown).

References

Arsimoles D, D'Esposito AG, Gaspoz V, *et al*. The AJ519 antibody labels the human TAC/IL2RA protein by immunofluorescence. Antibody Reports, 2020, 3:e118. doi:10.22450/journals/abrep.2020.e118

Jovčevska I, Zupanec N, Kočevar N, *et al*. TRIM28 and β-actin identified via nanobody-based reverse proteomics approach as possible human glioblastoma biomarkers. PLoS One. 2014; 9(11):e113688. PMID: 25419715

Lima WC. The AK423 antibody recognizes *Dictyostelium* actin network by immunofluorescence. Antibody Reports, 2019, 2:e54. doi:10.22450/ journals/abrep.2019.e54

Persson H, Ye W, Wernimont A, *et al*. CDR-H3 diversity is not required for antigen recognition by synthetic antibodies. J Mol Biol. 2013; 425(4):803-11. PMID: 23219464

Pollard TD. Actin and actin-binding proteins. Cold Spring Harb Perspect Biol. 2016; 8(8):a018226. PMID: 26988969

Victor KD, Pascual V, Williams CL, Lennon VA, Capra JD. Human monoclonal striational autoantibodies isolated from thymic B lymphocytes of patients with myasthenia gravis use VH and VL gene segments associated with the autoimmune repertoire. Eur J Immunol. 1992; 22(9):2231-6. PMID: 1516616

Conflict of interest

The authors declare no conflict of interest.

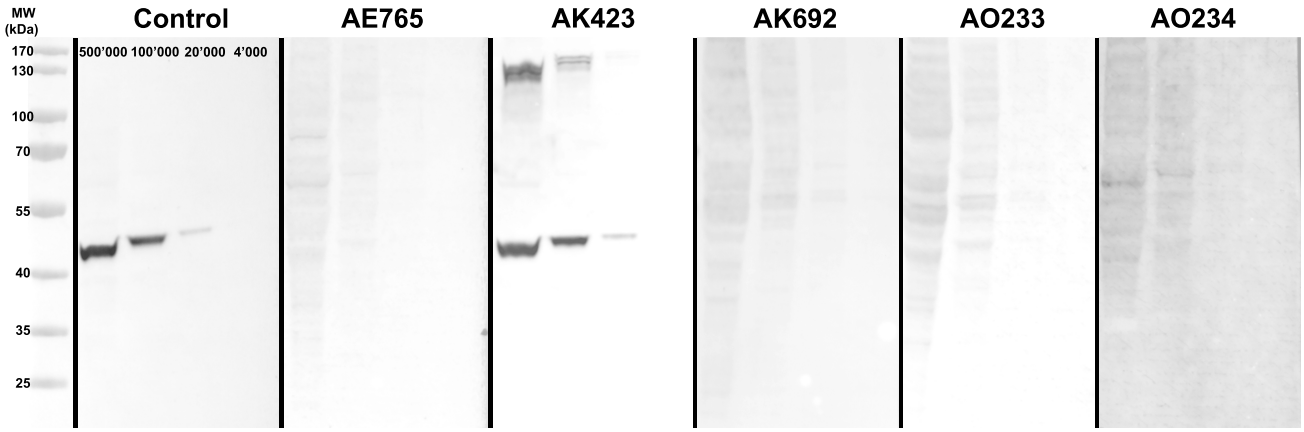

Fig. 1. The AK423 antibody specifically recognizes the actin protein of HEK cells. The same detection pattern is seen for the positive control, a commercial anti-beta actin antibody. AE765, AK692, AO233 and AO234 antibodies do not detect any specific bands. For each antibody, four sample dilutions were used: 500'000, 100'000, 20'000 and 4'000 cells per well (labels shown only for the first antibody, "Control").