The AK423 antibody recognizes Dictyostelium *and human actin with different affinities by western blot*

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Abstract

The AK423 antibody, raised against *Dictyostelium* actin, recognizes both *Dictyostelium* and human actin by western blot. However, it recognizes *Dictyostelium* actin with higher affinity than human actin.

Introduction

Actin is one of the most abundant proteins in eukaryotic cells, and a major structural component of the cytoskeleton. The AK423 antibody, originally raised against actin from the amoeba *Dictyostelium discoideum* (Uniprot P07830), also recognizes human actin by western blot, albeit with a lower binding affinity.

Materials & Methods

Antibodies: The ABCD_AK423 antibody (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as a mini-antibody (with the antigen-binding scFv fused to a rabbit IgG Fc; Lima, 2019), and as a double-chain mouse IgG. 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 IgG. Supernatants (10 mg/L for the scFv-Fc and 30 mg/L for the IgG) 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.

Antigen: HEK cells (grown in DMEM GlutaMAX™ (Gibco 31966) supplemented with 8% Fetal Bovine Serum (Gibco 10270)) and *Dictyostelium discoideum* DH1 cells (cultivated in HL5 medium) 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, 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 (final concentration 50 or 500 ng/mL in PBS-Tween). The membranes were washed three times (15+15+10 min) in PBS-Tween, incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad 170-6516, dilution 1:3000) or goat anti-rabbit IgG (Sigma-Aldrich A8275, dilution 1:3000) 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

AK423 is a recombinant antibody derived from the 224-236-1 hybridoma (Westphal *et al.*, 1997), raised against actin from the amoeba *Dictyostelium discoideum*. Previously, AK423 has been described as detecting actin from *Dictyostelium discoideum* (Lima, 2019) as well as human actin (Marchetti, 2022), albeit at a rather high concentration (5 µg/mL). A commercial anti-beta actin antibody, used as a positive control in previous experiments, is utilized at a much lower concentration (50 ng/mL; Marchetti, 2022).

We tested AK423 in two recombinant formats, as a mini-antibody (scFv fused to an Fc) and as a full IgG molecule, at the same concentration as the commercial antibody (50 ng/mL). At 50 ng/mL, AK423, in both formats, detected very efficiently *Dictyostelium* actin but not human actin (Fig. 1). The commercial antibody detected both *Dictyostelium* and human actin (Fig. 1). AK423 detected human actin only at a higher concentration (500 ng/mL) (Fig. 1).

As observed before (Marchetti, 2022), AK423 also cross-reacted with a band of higher molecular weight (~130-170 kDa) in human lysates (Fig. 1).

References

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Conflict of interest

The authors declare no conflict of interest

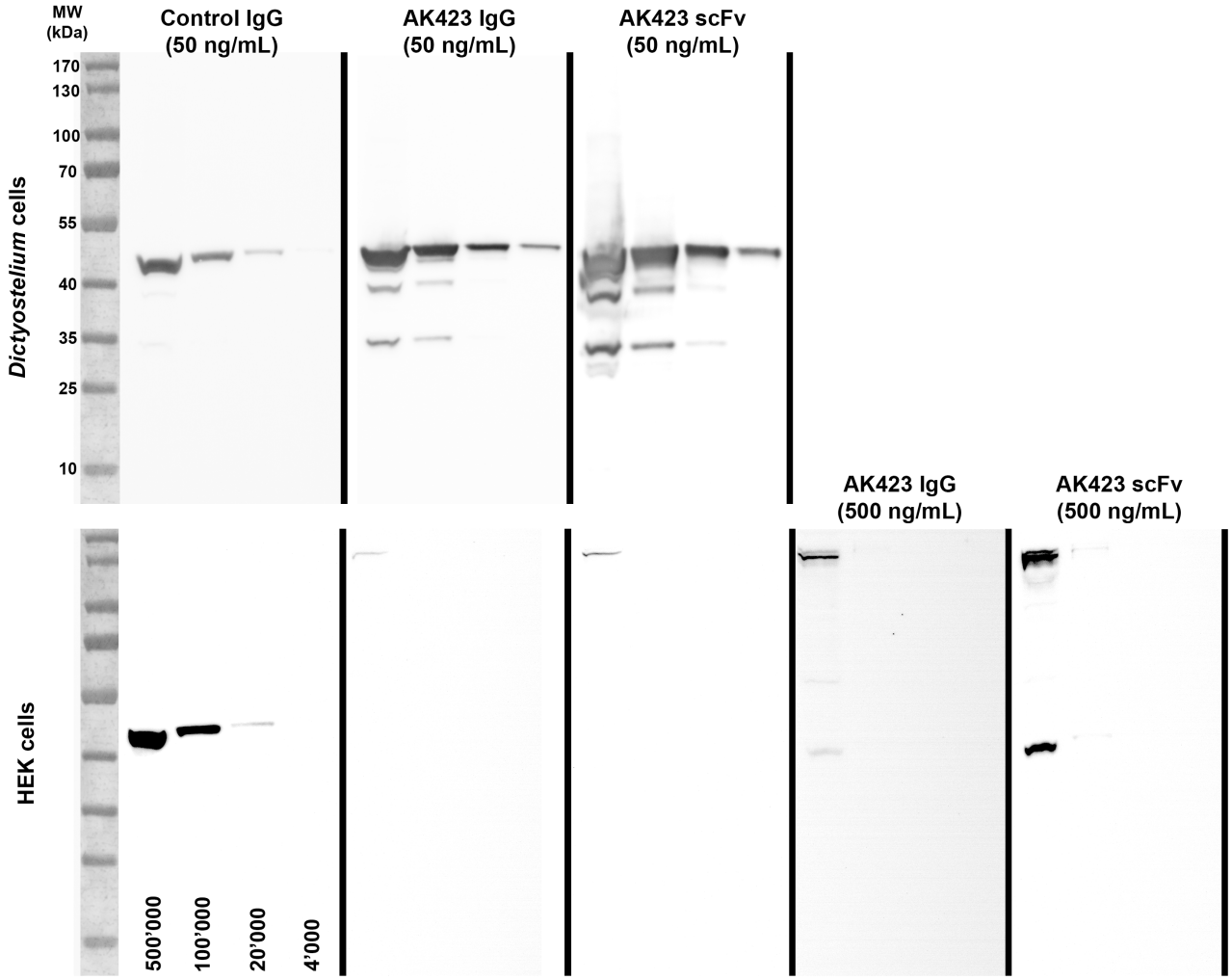

Fig. 1. The AK423 (at a final concentration of 50 ng/mL) recognizes the actin protein from *Dictyostelium* cells (predicted molecular mass ~42 kDa). At a higher concentration (500 ng/mL) it also recognizes human actin. The control IgG is a commercial anti-beta actin antibody from Proteintech. AK423 was tested in two different recombinant formats: as a full IgG molecule (AK423 IgG) and as a mini-antibody (AK423 scFv). For each antibody, four sample dilutions were used: 500'000, 100'000, 20'000 and 4'000 cells per well, as indicated for control antibody.