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The AK423 antibody recognizes Dictyostelium actin by Western blot

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Abstract

The AK423 antibody, derived from the 224-236-1 hybridoma, detects by Western blot the major actin protein from *Dictyostelium discoideum*.

Introduction

Actin is one of the most abundant proteins in eukaryotic cells, and a major structural component of the cytoskeleton. The major actin protein from *Dictyostelium* (UniProt #P07830) is encoded by 17 identical genes. Here we describe the ability of the AK423 antibody, a single chain fragment (scFv) derived from the 224-236-1 hybridoma (Westphal *et al*., 1997), to detect *Dictyostelium* actin by Western blot.

Materials & Methods

Antibodies: ABCD_AK423 antibody (ABCD nomenclature, web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibody with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions joined by a peptide linker $(GGGGS)_3$. The sequencing of the 224-236-1 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants were collected after 4 days; this antibody has a low production yield on our system (<10 mg/L).

Antigen: *D. discoideum* DH1 cells, cultivated in HL5 medium, were used to detect the full-length protein.

Protocol: 10^6 cells were pelleted, resuspended in 200 µL of 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), and boiled for 15 min at 95 ^{o}C. 10 µL of each sample ($5x10^4$ cells) was 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 30 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 during 1 hour at RT with either the 224-236-1 mouse monoclonal or the AK423 scFv antibody (diluted 1:10 or 1:20 in PBS-Tween, respectively). The membranes were then washed three times (5+5+15 min) and incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad#170-6516, dilution 1:3000) or anti-rabbit IgG (Sigma #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

Despite the fact the AK423 antibody is produced at low yield, it specifically recognizes the actin protein, detecting a single band around 42 kDa (Fig. 1), similarly to the original 224-236-1 hybridoma (Westphal *et al*., 1997).

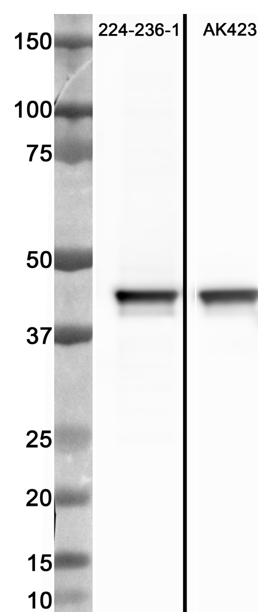

Fig. 1. The 224-236-1 hybridoma and the AK423 scFv specifically recognize the actin protein (predicted molecular mass ~42 kDa).

References

Westphal M, Jungbluth A, Heidecker M, *et al*. Microfilament dynamics during cell movement and chemotaxis monitored using a GFP-actin fusion protein. Curr Biol. 1997; 7(3):176-83. PMID:9276758

Conflict of interest

The authors declare no conflict of interest.

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