

# The AK421 antibody recognizes the *Dictyostelium* mitochondrial porin by immunofluorescence

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## Abstract

The AK421 antibody, derived from the 70-100-1 hybridoma, detects by immunofluorescence the mitochondrial porin from *Dictyostelium discoideum*.

## Introduction

The mitochondrial outer membrane porin (porA, DDB\_G0271848, UniProt #Q01501), also known as voltage-dependent anion-selective channel (VDAC), is a transmembrane protein that acts as a permeability channel for hydrophilic compounds (Troll *et al.*, 1992). Here we describe the ability of the AK421 antibody, a single chain fragment (scFv) derived from the 70-100-1 hybridoma, to label mitochondria by immunofluorescence.

## Materials & Methods

**Antibodies:** ABCD\_AK421 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 (GGGS)<sub>3</sub>. The sequencing of the 70-100-1 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 (~20 mg/L) were collected after 4 days.

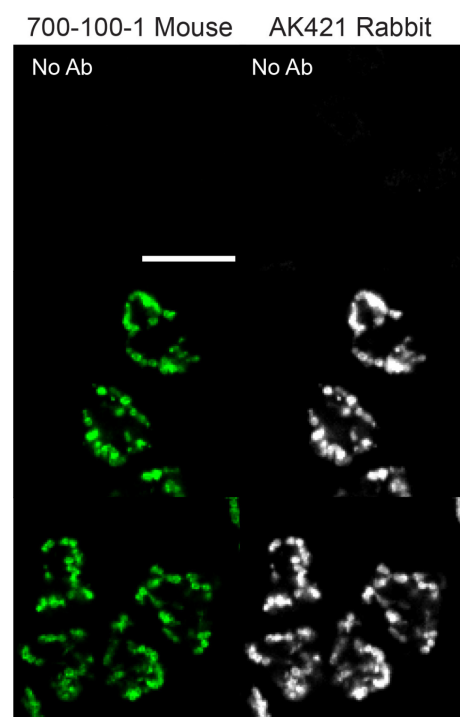
**Antigen:** 5x10<sup>5</sup> *D. discoideum* DH1 cells, sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 1 h at room temperature in HL5 medium, were used to detect the full-length protein.

**Protocol:** Cells were fixed with HL5 + 4% paraformaldehyde (w/v) (Appllichem, #A3013) for 30 min, then washed once in PBS for 5 min. Cells were then permeabilized in methanol at -20 °C for 2 min, washed once (5 min) with PBS, and blocked for 30 min with PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated for 30 min with the original mouse hybridoma 70-100-1 supernatant (dilution 1:3 in PBS-BSA) and with the reformatted AK421 scFv antibody (dilution 1:10 in PBS-BSA). After 3 washes (5, 5, 15 min) with PBS-BSA, cells were incubated for 45 min with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (hybridoma) and goat anti-rabbit IgG conjugated to AlexaFluor-647 (scFv) (1:300, Molecular Probes #A11029 and #A21245, respectively). After 3 washes (5, 5, 15 min) with PBS-BSA

and one wash (5 min) with PBS, coverslips were mounted on slides (Menzel-Gläser, 76x26 mm) with MÖwioI (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

In agreement with the original description of the 70-100-1 hybridoma (Troll *et al.*, 1992), the AK421 antibody labels mitochondria (Fig. 1). The staining with both antibodies appears almost indistinguishable (Fig. 1).



**Fig. 1.** The 700-100-1 hybridoma and the AK421 scFv label mitochondria in *Dictyostelium* cells. A double immunofluorescence staining with 700-100-1 and AK421 was performed. No labelling was seen when the primary antibodies were omitted (No Ab). Scale bar: 10  $\mu$ m.

## References

Troll H, Malchow D, Müller-Taubenberger A, *et al.* Purification, functional characterization, and cDNA sequencing of mitochondrial porin from *Dictyostelium discoideum*. *J Biol Chem.* 1992; 267:21072-9. PMID: 1328220

## Conflict of interest

Wanessa Cristina Lima is an editor of the Antibody Reports journal.