The AJ514 antibody recognizes the common antigen 1 from Dictyostelium discoideum by immunofluorescence

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Abstract
The AJ514 antibody, derived from the 221-342-5 hybridoma, detects by immunofluorescence the common antigen 1 from Dictyostelium discoideum.

Introduction
The common antigen 1 (CA1) is a mannose-6-SO4-carbohydrate epitope shared by lysosomal enzymes of Dictyostelium discoideum, recognized by the 221-342-5 monoclonal antibody (Knecht *et al*., 1984; Neuhaus *et al*., 1998). Here we describe the ability of the AJ514 antibody, a single chain fragment (scFv) derived from the 221-342-5 hybridoma, to label lysosomal compartments by immunofluorescence.

Materials & Methods
Antibodies: ABCD_AJ514 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 (GGGGSG). The sequencing of the 221-342-5 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 (~50 mg/L) were collected after 5 days.

Antigen: 5x10⁵ *D. discoideum* DH1 cells, sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 90 minutes at room temperature in HL5 medium, were used.

Protocol: Cells were fixed with HL5 + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH₄Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in methanol at -20 °C for 2 min, washed once (5 min) with PBS, and once (15 min) with PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated for 30 min with the original mouse hybridoma 221-342-5 supernatant (dilution 1:3 in PBS-BSA) and with the reformatted scFv antibody (dilution 1:10 in PBS-BSA). After 3 washes (5, 5, 15 min) with PBS-BSA, cells were incubated for 30 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 Mowiol (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 221-342-5 hybridoma antibody (Neuhaus *et al*., 1998), the AJ514 antibody labels intracellular organelles containing lysosomal enzymes exhibiting sulfated carbohydrates (Fig. 1). The staining with both antibodies appears almost indistinguishable (Fig. 1).

References


Conflict of interest
The authors declare no conflict of interest.
Fig. 1. The 221-342-5 hybridoma and the AJ514 antibody label lysosomal compartments in *Dictyostelium* cells. A double fluorescence staining with both antibodies was performed. No labelling was seen when the primary antibodies were omitted (No Ab). Scale bar: 10 µm.