

The AJ154 antibody recognizes the Dictyostelium p80 protein by immunofluorescence

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Abstract

The AJ154 antibody, derived from the H161 hybridoma, detects by immunofluorescence the full-length p80 protein from *Dictyostelium discoideum*.

Introduction

The p80 protein (DDB_G0287297, UniProt #Q7YXD4) is a widely-used marker for endosomal compartments in *D. discoideum*, recognized by the H161 monoclonal antibody (Ravel *et al.*, 2001). Here we describe the ability of the AJ154 antibody, a single chain fragment (scFv) derived from the H161 hybridoma, to label p80-endosomal compartments by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AJ154 antibody (ABCD nomenclature, <https://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 three different Fc moieties: mouse IgG2A, human IgG1 and rabbit IgG. 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 H161 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: *D. discoideum* DH1 (WT) cells were used to detect the full-length p80 protein.

Protocol: 10⁶ *D. discoideum* cells were sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 30 minutes at room temperature in HL5 medium. 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 (5 min) with PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated for 30 min with the original mouse hybridoma H161 supernatant (dilution 1:2 in PBS-BSA) or with each of the reformatted scFv antibodies (dilution 1:10 in PBS-BSA). After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-mouse, goat anti-rabbit or donkey anti-human IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes #A11029, #A11034 and Jackson ImmunoResearch #709-545-149, respectively) or, in the case of the H161 supernatant, with goat anti-mouse IgG conjugated to AlexaFluor-647 (1:300, Molecular Probes #A21235). After 3 washes (10 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 H161 hybridoma (Ravel *et al.*, 2001), the AJ154 antibody labels intracellular endocytic compartments (strongly staining post-lysosome compartments) and the cell surface (Fig. 1). The staining with AJ154 and H161 appears almost indistinguishable (Fig. 1A). Similar stainings were obtained with AJ154 exhibiting a mouse or a human Fc (Fig. 1B).

References

Ravel K, de Chassey B, Cornillon S, *et al.* Membrane sorting in the endocytic and phagocytic pathway of *Dictyostelium discoideum*. *Eur J Cell Biol.* 2001;80(12):754-64. PMID:11831389

Conflict of interest

The authors declare no conflict of interest.

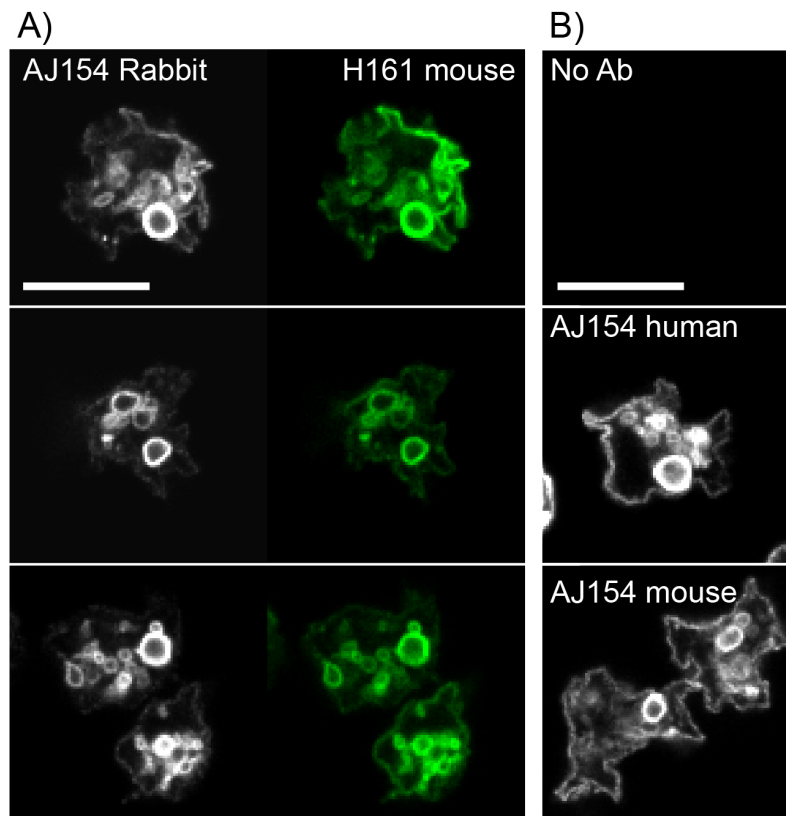


Fig. 1. The H161 hybridoma and the AJ154 antibody label endosomal compartments in *Dictyostelium* cells. In (A), a double fluorescence staining with AJ154 and the H161 hybridoma was performed. In (B), AJ154 fused to a human or a mouse Fc shows the same labelling pattern. No labelling was seen when the primary antibody was omitted (No Ab). Scale bar: 10 μ m.