

The AJ155 antibody recognizes the *Dictyostelium* p23 marker by immunofluorescence

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Abstract

The AJ155 antibody, derived from the H194 hybridoma, detects by immunofluorescence the p23-labelled compartments from *Dictyostelium discoideum*.

Introduction

The H194 monoclonal antibody recognizes the (unidentified) p23 antigen of *D. discoideum*, used as a marker of cell surface and intracellular compartments (Ravel *et al.*, 2001). Here we describe the ability of the AJ155 antibody, a single chain fragment (scFv) derived from the H194 hybridoma, to label p23 compartments by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AJ155 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 mouse IgG2A Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions joined by a peptide linker (GGGS)₃. The sequencing of the H194 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 H194 supernatant (dilution 1:3 in PBS-BSA) or 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) or AlexaFluor-647 (scFv) (1:300, Molecular Probes #A11029 and #A21235, 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 H194 hybridoma (Ravel *et al.*, 2001), the AJ155 antibody labels intracellular endocytic compartments and the cell surface (Fig. 1).

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.

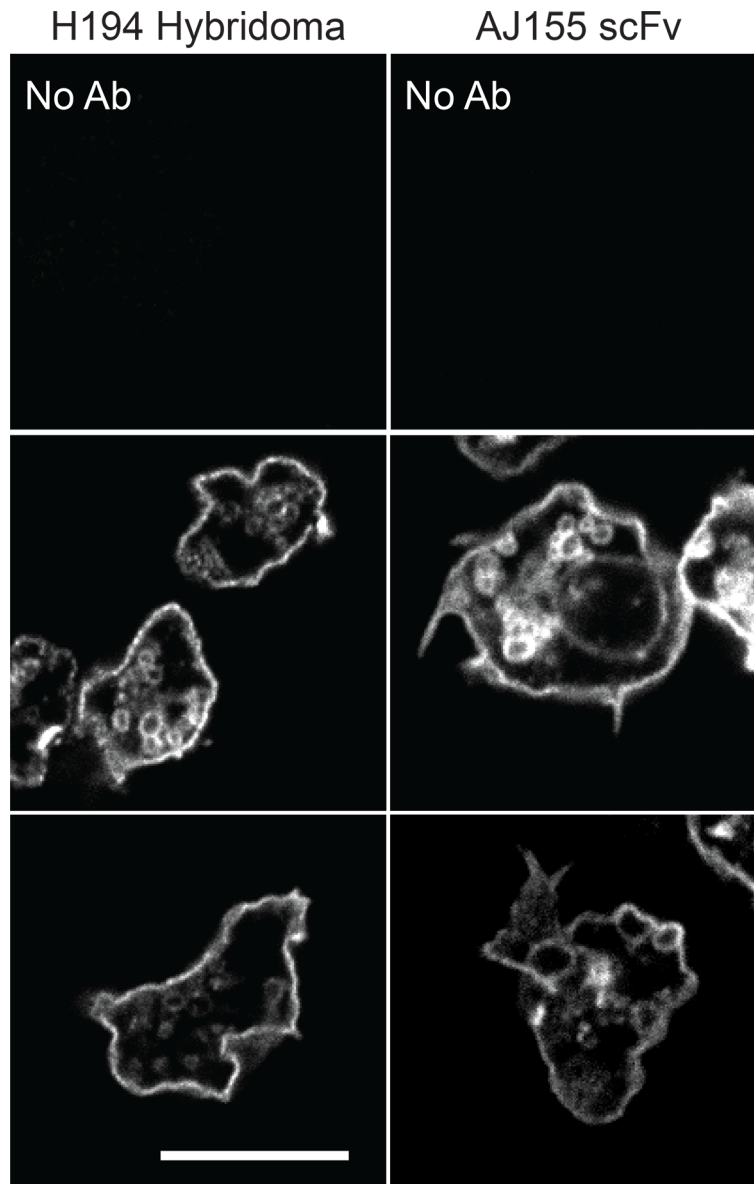


Fig. 1. The H194 hybridoma and the AJ155 antibody label the cell surface and endosomal compartments in *Dictyostelium* cells. No labelling was seen when the primary antibody was omitted (No Ab). Scale bar: 10 μ m.