

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

Pierre Cosson and Wanessa Cristina Lima are editors of the Antibody Reports journal.

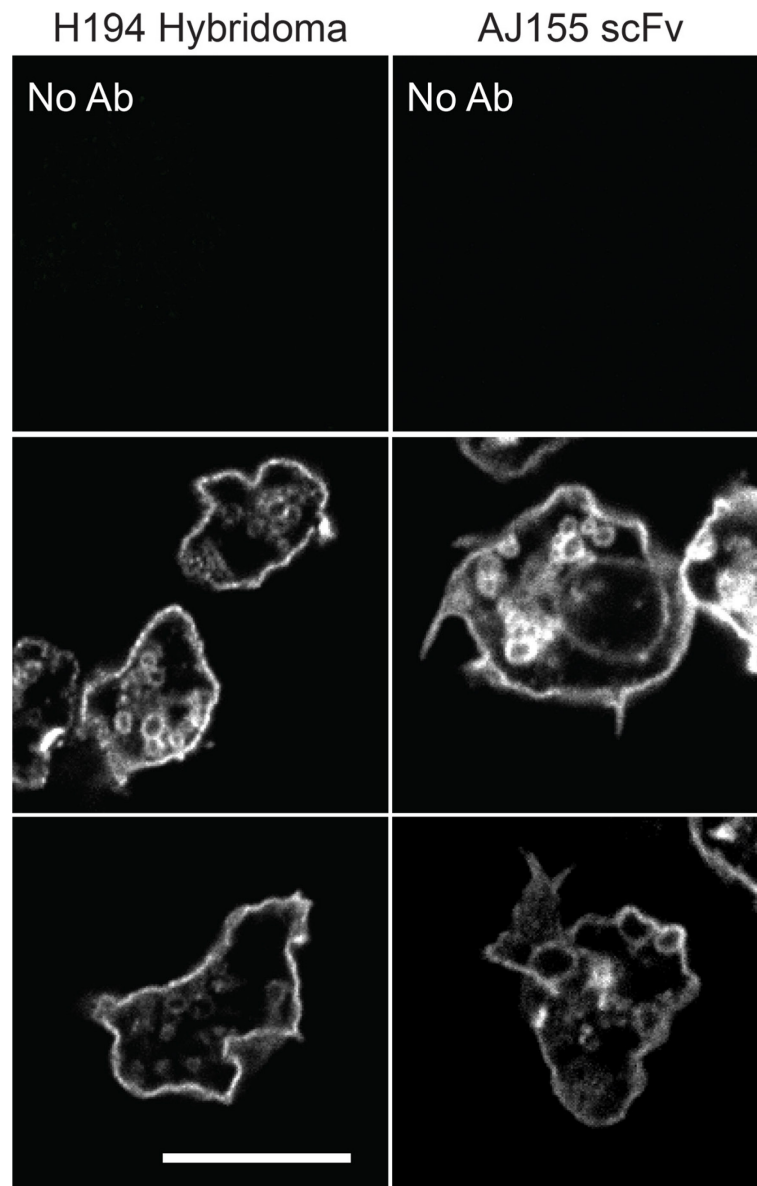


Fig. 1. The H194 hybridoma and the AJ155 antibody label the cell surface and endosomal compartments in *Dictyostelium* cells. No labelling was seen when the primary antibody was omitted (No Ab). Scale bar: 10 μ m.