The AN703 antibody recognizes Dictyostelium discoideum PDI by immunofluorescence

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Abstract
The AN703 antibody, derived from the 221-42-1 hybridoma, detects PDI-containing endoplasmic reticulum of Dictyostelium discoideum by immunofluorescence.

Introduction
Protein disulfide isomerase (PDI, DDB_G0276141, UniProt Q86IA3) is a protein present in the endoplasmic reticulum (ER) in D. discoideum, and it is recognized by the 221-42-1 monoclonal antibody (Monnat et al., 1997). Here we describe the ability of the AN703 antibody, a single chain fragment (scFv) derived from the 221-42-1 hybridoma, to label the endoplasmic reticulum by immunofluorescence.

Materials & Methods

Antibodies: The ABCD_AN703 antibody (ABCD nomenclature, http://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (http://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 (GGGGS). The sequencing of the 221-42-1 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. The supernatant (20 mg/L) was collected after 4 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 either (i) with HL5 + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, blocked with PBS + 40 mM ammonium chloride (NH₄Cl) (Applichem, #A3661) for 5 min, and then permeabilized in PBS + 0.1 % Triton X-100 for 5 min; or (ii) with methanol at -20 °C for 2 min. Cells were then washed once (1 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 221-42-1 supernatant (dilution 1:1 in PBS-BSA) or with the AN703 antibody (5 µg/ml 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 (1:400, Molecular Probes #A11029). 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 Möwiol (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
The AN703 antibody, like the 221-42-1 antibody (Monnat et al., 1997), labels the endoplasmic reticulum of Dictyostelium discoideum (Fig. 1). For both antibodies, cytosolic elements as well as a perinuclear staining are visible (Fig. 1).

References

Conflict of interest
The authors declare no conflict of interest.
Fig. 1. The 221-42-1 and the AN703 antibodies label the endoplasmic reticulum of Dictyostelium cells, using two different fixation/permeabilization methods. No labelling was seen when the primary antibody was omitted (No Ab). Scale bar: 10 µm.