The AJ072 antibody against the human transferrin receptor labels HeLa cells by surface immunofluorescence

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Abstract

The AJ072 antibody against the human transferrin receptor labels the cell membrane of HeLa cells by surface immunofluorescence; AM236 does not.

Introduction

TfR (Transferrin receptor protein 1, CD71; Uniprot P02786) is a type II transmembrane glycoprotein that binds the iron-carrier glycoprotein transferrin (Tf). Cellular uptake of iron occurs via receptor-mediated endocytosis of diferric Tf/TfR complexes (Candelaria *et al.*, 2021). Here, we describe the ability of the AJ072 recombinant antibody against human TfR1 to stain the cell membrane of HeLa cells by surface immunofluorescence; AM236 does not, presumably due to the fact that this antibody is poorly produced.

Materials & Methods

Antibodies: ABCD_AJ072 and ABCD_AM236 antibodies (https://web.expasy.org/abcd/, ABCD nomenclature) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the clones ch128.1 (for AJ072; Friden, 1994) and M16 (for AM236; Shusta and Tillotson, 2016) joined by a peptide linker $(GGGGS)_3$. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants were collected after 4 days; both antibodies have low production yields (<5 mg/L).

Antigen: HeLa cells were cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and grown in DMEM GlutaMAX™ (Gibco 31966) supplemented with 8% Fetal Bovine Serum (Gibco 10270).

Protocol: Cells were rinsed once with cold PBS, and kept for 30 min at 4 °C (ice+water bath). Cells were then incubated with the tested antibodies (undiluted, i.e. final concentration 5 mg/L in PBS + 0.2% (w/v) BSA (PBS-BSA)) for 15 min at 4 °C, fixed with PBS + 4% paraformaldehyde (w/v) (Applichem A3013) for 15 min at room temperature, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem A3661) for 5 min. After 1 wash (5 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes A11034). After 3 washes (10 min) with PBS-BSA, cells were incubated during 5 min with DAPI (1:500, Molecular Probes D1306), washed twice with PBS-BSA and once with PBS, and 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

Using a cell surface labeling protocol, AJ072 successfully labeled the plasma membrane of HeLa cells (Fig. 1). No staining was seen with AM236 antibody; this might be due to the fact that this antibody is poorly produced. No staining was observed when the primary antibody was omitted (Fig. 1, No Ab).

References

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Conflict of interest

The authors declare no conflict of interest.

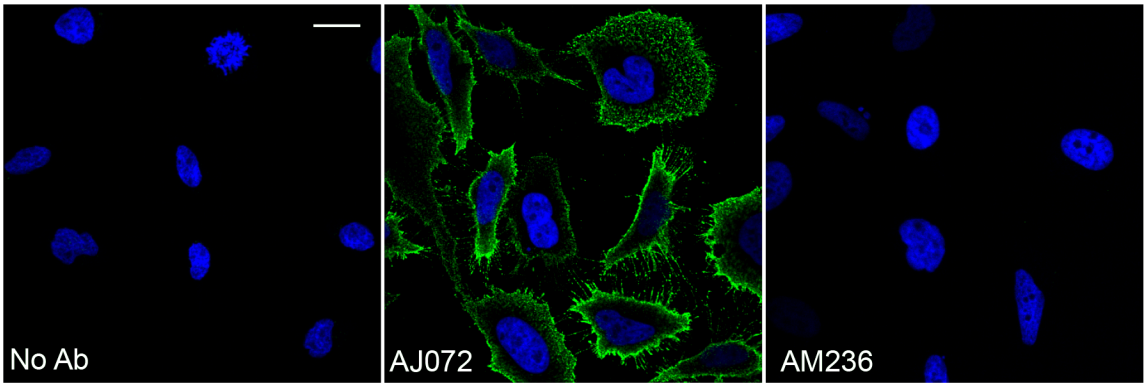

Fig. 1. AJ072 successfully labeled the cell membrane of HeLa cells (in green); in blue, nuclei were stained with DAPI. No labelling was seen for AM236 antibody, or when the primary antibody was omitted (No Ab panel). Scale bar: 20 μm.