

# The AJ072 antibody against the human transferrin receptor labels the surface and early endosomes of HEK cells by immunofluorescence

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## Abstract

The AJ072 antibody, against the human transferrin receptor, labels the plasma membrane and early endosomes of HEK cells by surface and total immunofluorescence.

## 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 TfR to stain the plasma membrane and early endosomes of HEK cells by surface and total immunofluorescence.

## Materials & Methods

**Antibodies:** Production of the AJ072 antibody, a mini-antibody with the antigen-binding scFv fused to a rabbit IgG Fc, has been described (Bian, 2022).

**Antigen:** HEK293T cells were cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and grown in DMEM GlutaMAX<sup>TM</sup> (Gibco 31966) supplemented with 10% Fetal Bovine Serum (Gibco 10270) and 100 µg/mL of penicillin-streptomycin (Gibco 15070-063) at 37 °C and 8% CO<sub>2</sub>.

**Protocol:** Cells were incubated with preheated DMEM GlutaMAX<sup>TM</sup> serum-free for 10 min at 37 °C. Cells were then incubated with transferrin AlexaFluor<sup>TM</sup>-546 conjugate (ThermoFisher T23364) at 5 µg/mL in DMEM GlutaMAX<sup>TM</sup> for 30 min at 37 °C. Then on ice, cells were rinsed thrice with cold PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated with undiluted AJ072 for 30 min at 4 °C, rinsed thrice with cold PBS-BSA and incubated for 30 min at 4 °C with secondary goat anti-rabbit IgG conjugated to AlexaFluor<sup>TM</sup>-488 (1:400, Thermo Fisher A11034). Cells were then rinsed thrice with cold PBS-BSA (3 min) and once with cold PBS (3 min). At room temperature, cells were then fixed with PBS + 4% paraformaldehyde (w/v) (Applichem A3013) for 30 min and blocked with PBS + 40 mM ammonium chloride

(NH<sub>4</sub>Cl) (Applichem A3661). Cells were then permeabilized in PBS containing 0.2% (w/v) saponin (Sigma S7900) for 5 min, washed once (3 min) with PBS-BSA, and incubated for 30 min with undiluted AJ072. After 3 washes (3 min) with PBS-BSA, cells were incubated for 30 min with secondary goat anti-rabbit IgG conjugated to AlexaFluor<sup>TM</sup>-647 (1:400, Thermo Fisher A21245), then washed thrice with PBS-BSA and once with PBS, and mounted on slides (Menzel-Gläser, 76x26 mm) with Mowiol (Hoechst) + 2.5% (w/v) DABCO (Fluka 33480). For the control cells, no primary antibody AJ072 was used. Pictures were taken using a Zeiss LSM800 Airyscan confocal microscope (Bioimaging Core Facility, Faculty of Medicine, University of Geneva), with a Plan-APO 40x/1.4 Oil DIC (UV) VIS-IR objective.

## Results

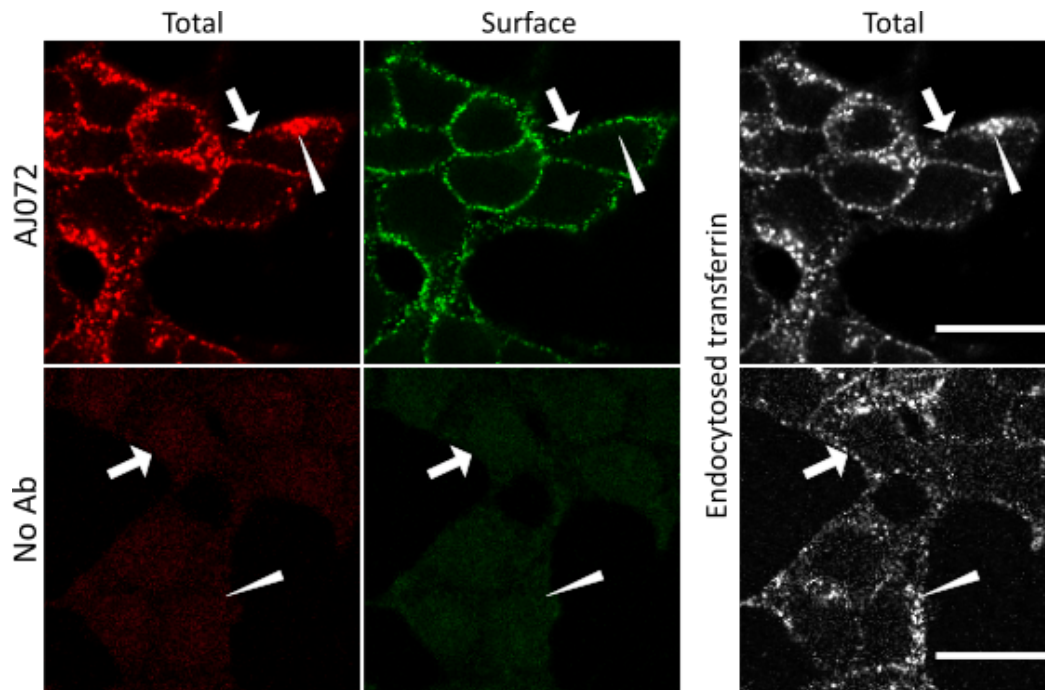
Using a cell surface labeling protocol, AJ072 successfully labeled the plasma membrane (Fig. 1, upper panel, green channel, arrows), as previously described (Bian, 2022). Using a total cell labeling protocol, AJ072 also labeled early endosomes in HEK cells (Fig. 1, upper panel, red channel, arrowheads), as indicated by co-localization with transferrin, a known marker for early endosomes (Fig. 1, upper panel, gray channel, arrowheads). No staining was observed when the primary antibody was omitted (Fig. 1, lower panels, green and red channels).

## References

- Bian C. The AJ072 antibody against the human transferrin receptor labels HeLa cells by surface immunofluorescence. *Antibody Reports*, 2022, 5:e644. doi:10.24450/journals/abrep.2022.e644.
- Candelaria PV, Leoh LS, Penichet ML, Daniels-Wells TR. Antibodies targeting the Transferrin Receptor 1 (TfR1) as direct anti-cancer agents. *Front Immunol.* 2021; 12:607692. PMID: 33815364.

## Conflict of interest

The authors declare no conflict of interest.



**Fig. 1.** AJ072 successfully labeled the cell membrane (green channel, arrows) of HEK cells by surface immunofluorescence. AJ072 also successfully labeled the plasma membrane (arrows) and early endosomes (arrowheads) of HEK cells by total immunofluorescence. Early endosomes and cell surface were stained with surface-bound and endocytosed transferrin Alexa Fluor™-546 (in gray). The AJ072 intracellular labeling co-localized with the endocytosed transferrin (arrowheads). No labeling was observed when the primary antibody was omitted (No Ab panels). Scale bar: 20  $\mu$ m.