

The AF161 and AF300 antibodies against human cytokeratin recognize the cytoskeleton by immunofluorescence in HeLa cells

Wanessa Cristina Lima, Pierre Cosson

Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The AF161 and AF300 antibodies detect human cytokeratin in methanol-fixed HeLa cells.

Introduction

Cytokeratins, a diverse family of proteins expressed by different human tissues, are important components of cytoskeletal intermediate filaments (Moll *et al.*, 1982). Two recombinant antibodies, AF161 targeting human cytokeratins 8/18 (Uniprot #P05787 and #P05783) and AF300 targeting human cytokeratin 17 (Uniprot #Q04695), reveal a cytoskeleton-like pattern in human HeLa cells by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AF161 and ABCD_AF300 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) were produced by the Geneva Antibody Facility (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 COU-1 (for AF161; Ditzel *et al.*, 1997) and A4 (for AF300; Wang *et al.*, 2004) joined by a peptide linker (GGGS)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; while AF161 produces well (80 mg/L), AF300 has a low production yield on our system (<5 mg/L).

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

Protocol: The whole procedure was carried out at room temperature. Cells were rinsed once with PBS, and fixed with methanol at -20 °C for 3 min. Fixed cells were washed once in PBS and once with PBS + 0.2% (w/v) BSA (PBS-BSA) during 5 min, and incubated for 30 min with each of the tested antibodies (final concentration 5 mg/L in PBS-BSA). After 3 washes (10 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

AF161 and AF300 antibodies successfully detect cytoskeletal filaments in HeLa cells (Fig. 1). The differences between the stainings observed with AF161 and AF300 suggest different intracellular localizations for different cytokeratins, but this was not investigated further here. No staining is observed when the primary antibody is omitted (Fig. 1, No Ab).

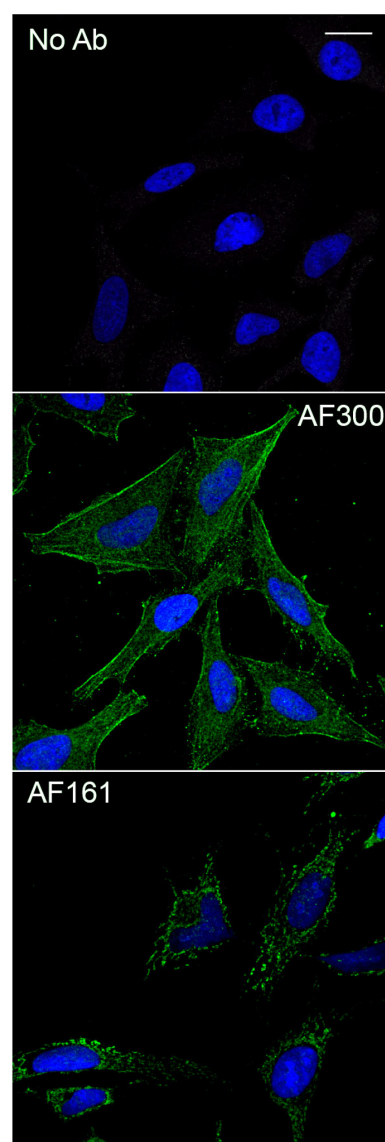


Fig. 1. AF161 and AF300 antibodies successfully label the cytoskeletal filaments in HeLa cells (in green); in blue, nuclei are stained with DAPI. No labelling is seen when the primary antibody is omitted (No Ab panel). Scale bar: 20 µm.

References

- Ditzel HJ, Garrigues U, Andersen CB, *et al.* Modified cytokeratins expressed on the surface of carcinoma cells undergo endocytosis upon binding of human monoclonal antibody and its recombinant Fab fragment. *Proc Natl Acad Sci USA.* 1997; 94(15):8110–8115. PMID:9223323
- Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. *Nucleic Acids Res.* 2019; pii:gkz714. PMID:31410491
- Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell.* 1982; 31(1):11-24. PMID:6186379.
- Wang G, Liu YF, Li CY, *et al.* Cloning and characterization of antikeratin human antibodies using a semisynthetic phage antibody library. *Arch Dermatol Res.* 2004; 296(6):270–277. PMID:15340746

Conflict of interest

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