The AA344 and AA345 antibodies detect human tubulin by immunofluorescence in HeLa cells

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Abstract

The AA344 and AA345 antibodies detects by immunofluorescence human tubulin in methanol-fixed HeLa cells.

Introduction

Tubulin, the major microtubule component, is a dimer of alpha- (TUBA, UniProt #P68363) and beta-tubulin (TUBB, Uniprot #P07437). Here we describe the ability of two recombinant antibodies (AA344 and AA345) to successfully label microtubules by immunofluorescence in human HeLa cells; AG890 does not.

Materials & Methods

Antibodies: ABCD_AA344, ABCD_AA345, and ABCD_AG890 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 mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$. AA344 (clone S11B; Nizak *et al.*, 2003) and AG890 (clone FabA1; Correa *et al.*, 2013) detect beta-tubulin; AA345 (clone F2C; Nizak *et al.*, 2003) detects alpha-tubulin. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (70, 100, and 90 mg/L, for AA344, AA345, and AG890 respectively) were collected after 4 days.

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-mouse IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes #A11029). 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

AA344 and AA345 antibodies successfully label a network of cytoskeletal filaments typical of the microtubule cytoskeleton in HeLa cells (Fig. 1). AG890 does not label any cellular structure. No staining is observed when the primary antibody was omitted (Fig. 1, No Ab panel).

References

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Conflict of interest

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

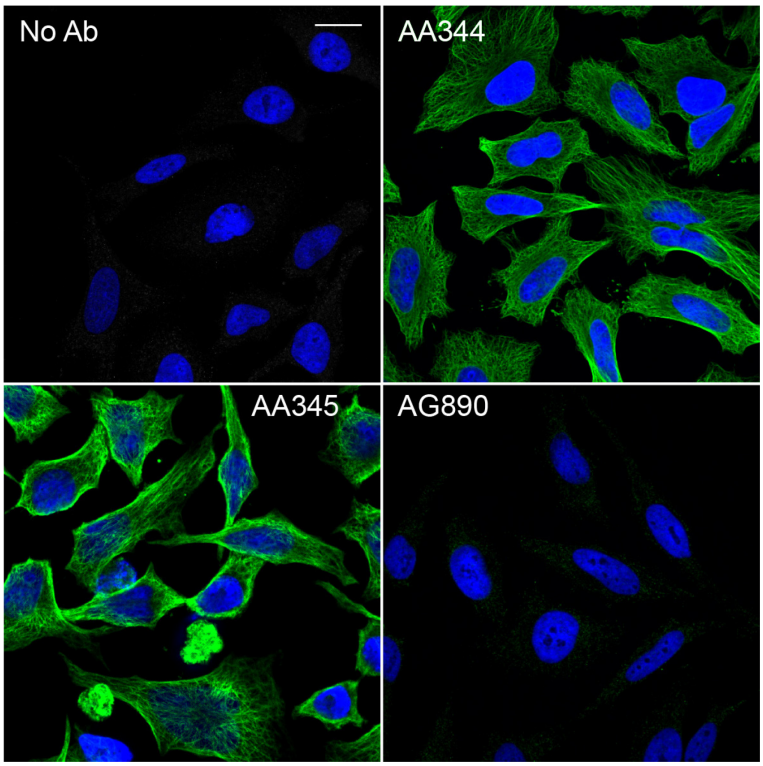

Fig. 1. AA344 and AA345 antibodies successfully label the microtubule network in HeLa cells (in green); in blue, nuclei are stained with DAPI. No labelling is seen when the primary antibody is omitted (No Ab panel). Scale bar: 20 μm.