

AA344 and AA345 antibodies recognize the microtubule network in human cells by immunofluorescence

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Abstract

The recombinant antibodies AA344 and AA345 detect the microtubule network by immunofluorescence in interphase and mitotic glutaraldehyde-fixed Retinal Pigment Epithelial cells (hTert-RPE1).

Introduction

Microtubules are highly dynamic polymers, composed of α/β -tubulin dimers that switch between periods of growth and shrinkage fueled by GTP hydrolysis. Microtubules are an essential part of the cell cytoskeleton that provide tracks for intracellular transport, and contribute to cell shape, mobility and organization. During cell division, microtubules assemble the mitotic spindle that will attach and segregate the chromosomes. Here we describe the antibodies AA344 and AA345, which recognize human β - and α -tubulin respectively (Nizak *et al.*, 2003). We show how in interphase and mitotic human RPE1 cells both antibodies detect the microtubule network by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AA344 and ABCD_AA345 antibodies (ABCD nomenclature, <https://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies; Blanc *et al.*, 2014) as mini-antibodies with the antigen-binding scFv portion fused to a human IgG1 Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond, respectively, to the sequence of the variable regions of the clones S11B and F2C (Nizak *et al.*, 2003) 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 of each antibody. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: hTert-RPE1 cells, non-transformed human retina pigment epithelial cells immortalized with telomerase, were cultured on a glass coverslip (Menzel-Gläser, 22x22 mm) and grown in Dulbecco's modified medium (DMEM) supplemented with 10% FCS and 100 U/ml penicillin and 100 mg/ml streptomycin, at 37 °C with 5% CO₂ in a humidified incubator.

Protocol: Cells were rinsed with cytoskeleton buffer (CB; 10 mM MES, 150 mM NaCl₂, 5 mM MgCl₂, 5 mM glucose; Sigma Aldrich), then fixed with a glutaraldehyde solution in CB (0.05% glutaraldehyde, 3% formaldehyde, and 0.1% Triton X; Sigma Aldrich) for 15 min at RT, and rinsed 2 x 10 min with CB. Cells were incubated 1 h with the primary antibody (diluted 1:50) in a solution containing PBS with 3% BSA and 1% sodium azide (N₃Na) (Applichem, #1430). After 3 x 10 min washes using PBS with 0.05% Tween-20, cells were incubated 30 min with secondary goat anti-human IgG conjugated to AlexaFluor-488 (1:400, Invitrogen #A11013). After 3x10 min washes using PBS with 0.05% Tween-20, coverslips were mounted on slides (Menzel-Gläser, 76x26 mm) with DAPI Vectashield mounting medium (Vector Laboratories, #H-1200) formerly cleaned with 70% ethanol. Pictures were acquired in 0.2 μ m steps using 100x NA 1.4 objectives on Olympus DeltaVision microscope (GE Healthcare, Switzerland) equipped with a DAPI/FITC/TRITC/CY5 filter set (Chroma, Bellow Falls, VT) and a CoolSNAP HQ camera (Roper Scientific, Tuscon USA). 3D image stacks were deconvolved with SoftWorx (GE Healthcare). Images were analyzed with Image J and mounted in figures using Adobe Illustrator.

Results

Antibodies AA344 and AA345 specifically detected microtubules in interphase and metaphase hTert-RPE1 cells after a glutaraldehyde-based fixation (Fig. 1A and B). In interphase the antibodies stained the radial microtubule network emanating from the centrosome (Fig. 1A); in mitosis the antibodies stained the mitotic spindle and astral microtubules (Fig. 1B). No signal was detected when the primary antibody was omitted.

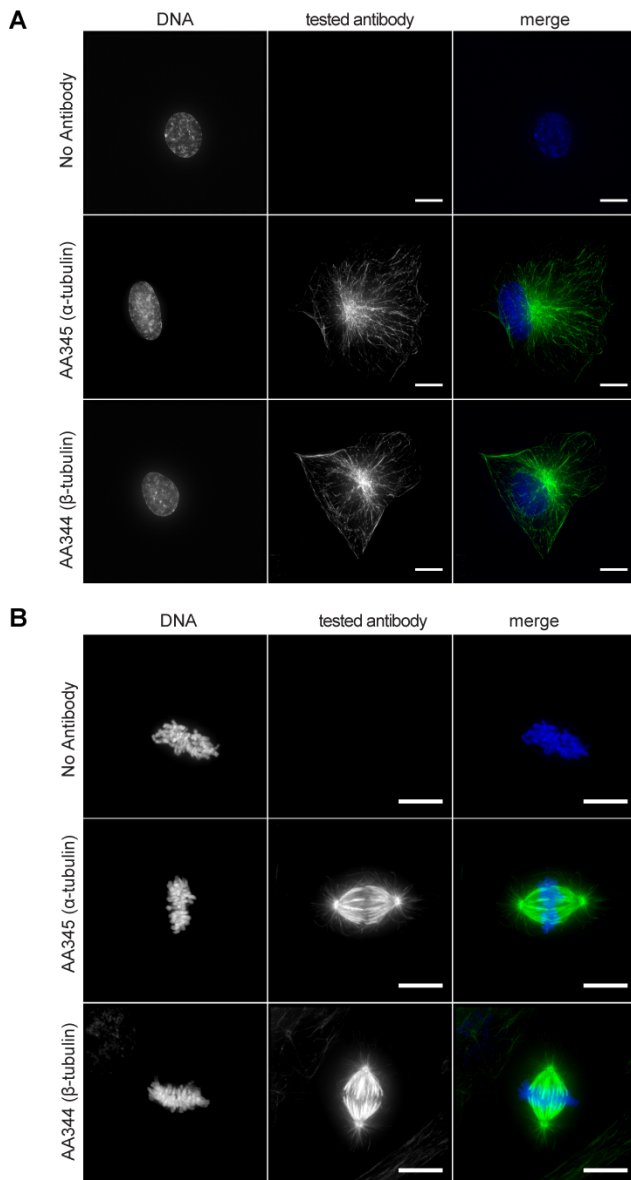


Fig. 1. AA344 and AA345 antibodies stain the microtubule network in interphase (A) and metaphase (B) hTert-RPE1 cells. Cells were stained either with no antibodies (top), AA345 (middle) or AA344 (bottom; all in green) and DAPI (DNA; blue). Scale bar: 10 μ m.

References

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Conflict of interest

The authors declare no conflict of interest.