

# The AA345 antibody specifically recognizes $\alpha$ -tubulin by western blot in human cell lines

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## Abstract

The recombinant antibody AA345 specifically detects human  $\alpha$ -tubulin in HEK293, Hela, and HepG2 human cell lines by western blot.

## Introduction

The recombinant AA345 antibody was previously shown to successfully recognize the human  $\alpha$ -tubulin by immunofluorescence (Guerreiro and Meraldi, 2019; Lima and Cosson, 2019). Here, we add a new application to the AA345 antibody by describing its ability to detect the human  $\alpha$ -tubulin in cell lysates by western blot.

## Materials & Methods

**Antibodies:** ABCD\_AA345 antibody (ABCD nomenclature, <https://web.expasy.org/abcd/>) was produced by the Geneva Antibody Facility (<https://www.unige.ch/medecine/antibodies/>) as a mini-antibody with the antigen-binding scFv fused to a mouse IgG2a Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequences of the variable regions of the clone F2C (Nizak *et al.*, 2003) joined by a peptide linker (GGGS)<sub>3</sub>. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (~100 mg/L) were collected after 4 days.

**Cell culture and lysis:** HEK293 and Hela cells were grown in high glucose DMEM without pyruvate (Gibco, #41965-039) supplemented with 10% FBS. HepG2 cells were grown in low glucose DMEM with pyruvate (Gibco, #31885-023) supplemented with 10% FBS. Cells were seeded in 6-well plates and cultured to reach 70-80% confluency. Total cellular proteins were extracted by lysing cells in 200  $\mu$ l RIPA buffer (10 mM Tris pH 7.4, 150 mM NaCl, 0.1% SDS, 1X Triton, 2 mM EDTA). Lysates were sonicated and proteins were quantified using Pierce™ BCA protein assay kit (Thermo Scientific, #23227).

**Protein separation and transfer:** Cell lysates containing 20  $\mu$ g of total protein were mixed with 4X NuPAGE® LDS sample buffer (Thermo Fisher Scientific, #NP0007) and 0.1 M DTT. Samples were heated at 70 °C for 10 min prior to loading in duplicate onto a Bolt™ 4-12% Bis-Tris pre-casted gel (Thermo Fisher Scientific,

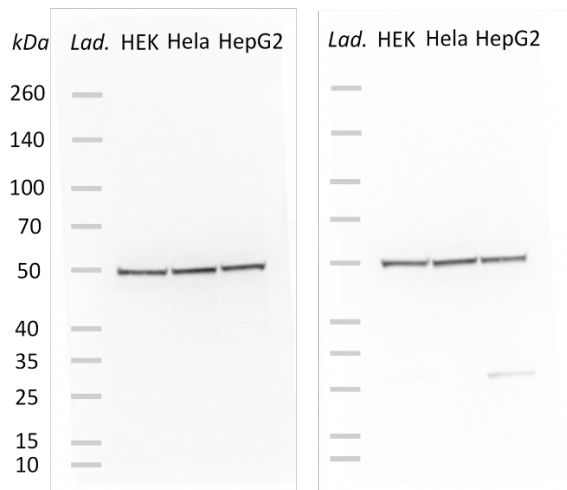
#NW04120BOX) and run at 150 V for 1 h along with Spectra™ Multicolor Broad Range Protein ladder (Thermo Scientific, #26634). In the meantime, a 0.45  $\mu$ m PVDF membrane (Millipore, #IPVH85R) was activated in Isopropanol for 30 s and further soaked in transfer buffer (25 mM Tris, 186 mM Glycine, 20% v/v Isopropanol) along with blot paper (Bio-Rad, #1703966). After complete protein migration, the gel was equilibrated in transfer buffer for 5-10 min. Protein transfer was performed using a Trans-Blot® SD semi-dry transfer cell (Bio-Rad, #1703848) at 300 mA for 15 min.

**Immunoblotting:** The membrane was cut in half. Both pieces were rinsed with water and blocked with 5% BSA in TBS (Tris 20 mM, NaCl 150 mM, pH 7.6) for 1 h. Membranes were washed 2 times for 10 min in TBS-Tween 0.1% and incubated overnight at 4 °C with anti- $\alpha$ -tubulin primary antibodies AA345 or T5168 (Merck; Piperno *et al.*, 1987) diluted to 1:5000 and 1:7500, respectively, in TBS-Tween 0.1% containing 5% BSA. On the next day, membranes were washed 3 times for 10 min with TBS-Tween 0.1% and incubated with HRP-conjugated anti-mouse secondary antibody (Merck, #A5278) for 1 h at RT.

**Protein detection:** Membranes were washed 3 times for 5 min with TBS-Tween 0.1% and antibody-bound protein bands were visualized by the addition of SuperSignal™ West Pico PLUS chemiluminescent substrate (Thermo Scientific, #34580) directly on the membranes placed in the iBRIGHT Western Blot imaging system (Thermo Fisher Scientific, #FL1500). Membranes were exposed for 10 s, 30 s, and 1 min until signal saturation.

## Results

The recombinant antibody AA345 successfully and specifically detected human  $\alpha$ -tubulin (50 kDa) in each of the tested cell lines (Fig. 1). It did so with a specificity superior to the commercial monoclonal anti- $\alpha$ -tubulin used as a control (T5168), which shows lower unspecific bands in both HEK293 and HepG2 cells.



**Fig. 1.** Specific detection of human  $\alpha$ -tubulin by western blot (predicted molecular weight: 50 kDa in reducing conditions) in lysates of HEK293, HeLa, and HepG2 cells using antibodies AA345 (left) and control T5168 (right). Detection of chemiluminescence signal upon 30 s (AA345, left) or 10 s (T5168, right) of exposure.

## References

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## Conflict of interest

The authors declare no conflict of interest.