Anti-beta Tubulin Antibody [1-B11]

EM0103

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	1-B11

Description: Tubulin in molecular biology can refer either to the tubulin protein superfamily of globular proteins, or one of the member proteins of that superfamily. α- and β-tubulins polymerize into microtubules, a major component of the eukaryotic cytoskeleton. Microtubules function in many essential cellular processes, including mitosis. Tubulin-binding drugs kill cancerous cells by inhibiting microtubule dynamics, which are required for DNA segregation and therefore cell division. In eukaryotes, there are six members of the tubulin superfamily, although not all are present in all species. Both α and β tubulins have a mass of around 50 kDa and are thus in a similar range compared to actin (with a mass of ~42 kDa). In contrast, tubulin polymers (microtubules) tend to be much bigger than actin filaments due to their cylindrical nature. Tubulin was long thought to be specific to eukaryotes. More recently, however, several prokaryotic proteins have been shown to be related to tubulin.

Immunogen: Synthetic peptide within human Beta tubulin aa 51-100.

Positive control: K-562 cell lysate, Jurkat cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, C6 cell lysate, Hela, HepG2, NIH/3T3, mouse brain tissue, hybrid fish (crucian-carp) brain tissue lysates.

Subcellular location: Cytoplasm ,cytoskeleton

Database links: SwissProt: P07437 Human | P99024 Mouse | P69897 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IF-Cell	1:1,00
IHC-P	1:3,000-1:10,000
FC	1:50-1:100
IF-Tissue	1:600-1:2,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

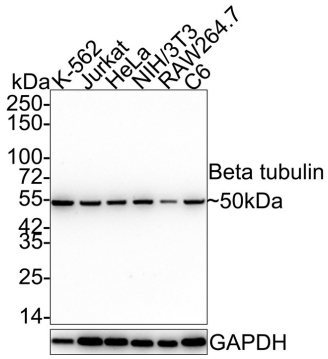


Fig1: Western blot analysis of beta Tubulin on different lysates with Mouse anti-beta Tubulin antibody (EM0103) at 1/20,000 dilution.

Lane 1: K-562 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: HeLa cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: RAW264.7 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 50 kDa
Observed band size: 50 kDa

Exposure time: 1 minute 40 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM0103) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:50,000 dilution was used for 1 hour at room temperature.

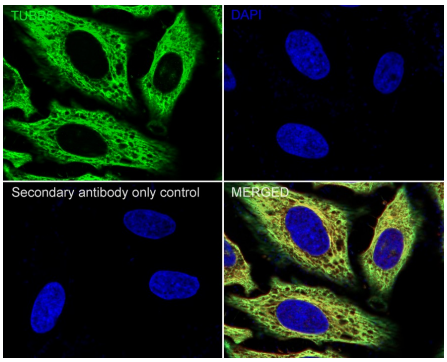


Fig2: Immunocytochemistry analysis of HeLa cells labeling beta Tubulin with Mouse anti-beta Tubulin antibody (EM0103) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-beta Tubulin antibody (EM0103) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

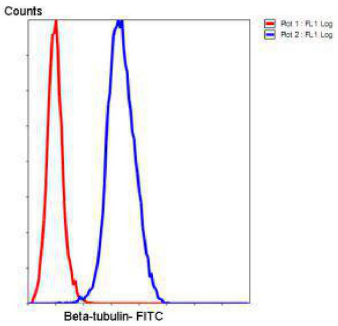


Fig3: Flow cytometric analysis of Hela cells with β-tubulin antibody at 1/50 dilution (blue) compared with an unlabelled control (cells without incubation with primary antibody; red). Goat anti mouse IgG (FITC) was used as the secondary antibody.

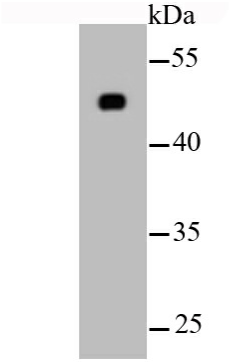


Fig4: Western blot analysis of β-tubulin on hybrid fish (crucian-carp) brain tissue lysate using anti-β-tubulin antibody at 1/500 dilution.

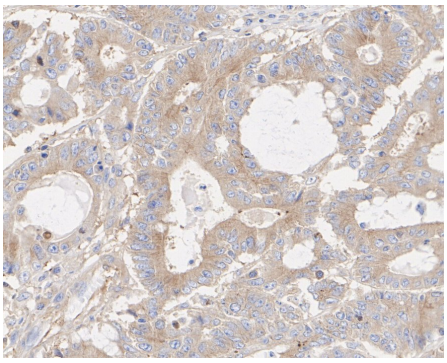


Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Mouse anti-beta Tubulin antibody (EM0103) at 1/3,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM0103) at 1/3,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

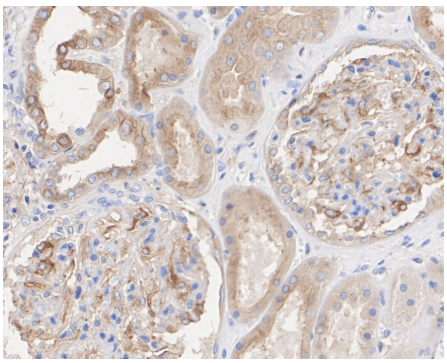


Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-beta Tubulin antibody (EM0103) at 1/3,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM0103) at 1/3,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

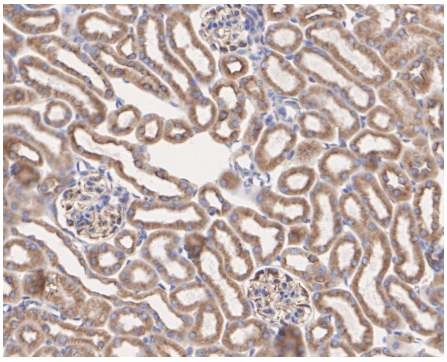


Fig7: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Mouse anti-beta Tubulin antibody (EM0103) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM0103) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

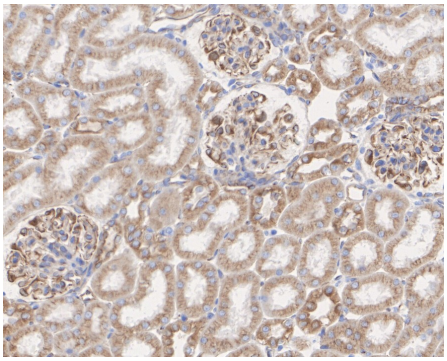
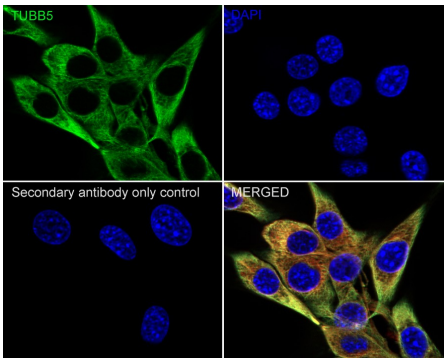


Fig8: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Mouse anti-beta Tubulin antibody (EM0103) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM0103) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig9: Immunocytochemistry analysis of NIH/3T3 cells labeling beta Tubulin with Mouse anti-beta Tubulin antibody (EM0103) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-beta Tubulin antibody (EM0103) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

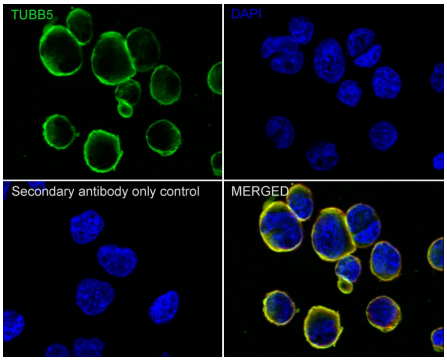


Fig10: Immunocytochemistry analysis of PC-12 cells labeling beta Tubulin with Mouse anti-beta Tubulin antibody (EM0103) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-beta Tubulin antibody (EM0103) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

Background References

1. "Evolutionary history of a multigene family: an expressed human beta-tubulin gene and three processed pseudogenes." Lee M.G.-S., Lewis S.A., Wilde C.D., Cowan N.J. Cell 33:477-487(1982)
2. "Tubulins in the primate retina: evidence that xanthophylls may be endogenous ligands for the paclitaxel-binding site." Crabtree D.V., Ojima I., Geng X., Adler A.J. Bioorg. Med. Chem. 9:1967-1976(2000)
3. "Mutations in the beta-tubulin gene TUBB5 cause microcephaly with structural brain abnormalities." Breuss M., Heng J.I., Poirier K., Tian G., Jaglin X.H., Qu Z., Braun A., Gstrein T., Ngo L., Haas M., Bahi-Buisson N., Moutard M.L., Passemard S., Verloes A., Gressens P., Xie Y., Robson K.J., Rani D.S. Cell Rep. 2:1554-1562(2011)