Anti-Beta III Tubulin Antibody [SP06-00]

ET1604-17

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IP, FC, IHC-Fr, IF-Cell
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	SP06-00

Description: Tubulin is a major cytoskeleton component that has five distinct forms, designated α, β, γ, δ and e Tubulin. α and β Tubulins form heterodimers which multimerize to form a microtubule filament. Multiple β Tubulin isoforms (β1, β2, β3, β4, β5, β6 and β8) have been characterized and are expressed in mammalian tissues. β1 and β4 are present throughout the cytosol, β2 is present in the nuclei and nucleoplasm, and β3 is a neuron-specific cytoskeletal protein. γ Tubulin forms the gammasome, which is required for nucleating microtubule filaments at the centrosome. Both δ Tubulin and e Tubulin are associated with the centrosome. δ Tubulin is a homolog of the Chlamydomonas δ Tubulin Uni3 and is found in association with the centrioles, whereas e Tubulin localizes to the pericentriolar material. e Tubulin exhibits a cell-cycle-specific pattern of localization, first associating with only the older of the centrosomes in a newly duplicated pair and later associating with both centrosomes.

Immunogen: Synthetic peptide within Human Tubulin beta-III aa 392-441 / 450.

Positive control: SH-SY5Y cell lysate, HeLa cell lysate, Neuro-2a cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, C6 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, SH-SY5Y, Neuro-2a, PC-12, mouse hippocampus (DG) tissue, mouse hippocampus (CA1) tissue, mouse brain tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: Q13509 Human | Q9ERD7 Mouse | Q4QRB4 Rat

Recommended Dilutions:

WB	1:20,000
IHC-P	1:50-1:200
FC	1:100
IP	1-2μg/sample
IHC-Fr	1:100
IF-Cell	1:100-1:200

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

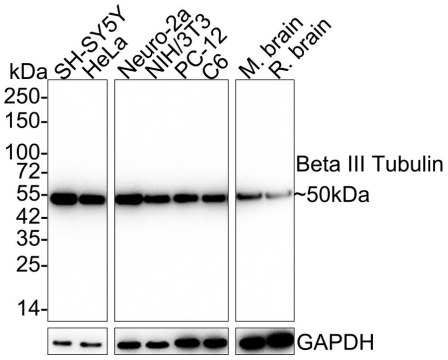

Fig1: Western blot analysis of Beta III Tubulin on different lysates with Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/20,000 dilution.

Lane 1: SH-SY5Y cell lysate (15 µg/Lane)
Lane 2: HeLa cell lysate (15 µg/Lane)
Lane 3: Neuro-2a cell lysate (15 µg/Lane)
Lane 4: NIH/3T3 cell lysate (15 µg/Lane)
Lane 5: PC-12 cell lysate (15 µg/Lane)
Lane 6: C6 cell lysate (15 µg/Lane)
Lane 7: Mouse brain tissue lysate (20 µg/Lane)
Lane 8: Rat brain tissue lysate (20 µg/Lane)

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

Exposure time: 3 minutes 54 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 (ET1604-17) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

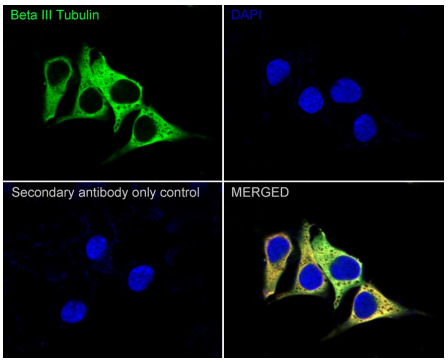

Fig2: Immunocytochemistry analysis of SH-SY5Y cells labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/100 dilution.

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 Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

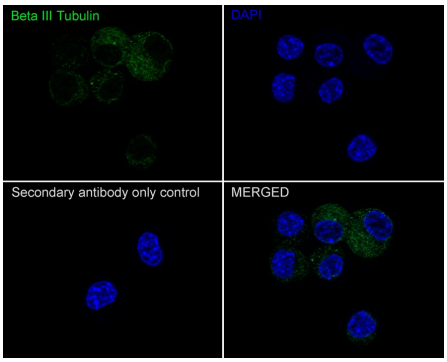

Fig3: Immunocytochemistry analysis of Neuro-2a cells labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/200 dilution.

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 Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/200 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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.

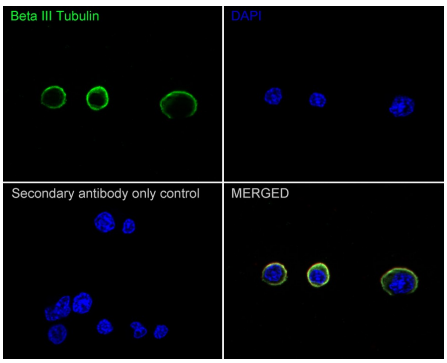

Fig4: Immunocytochemistry analysis of PC-12 cells labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/100 dilution.

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 Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

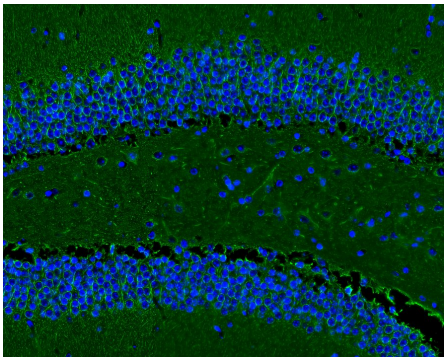

Fig5: Immunofluorescence analysis of frozen mouse hippocampus (DG) tissue labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (ET1604-17).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1604-17, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

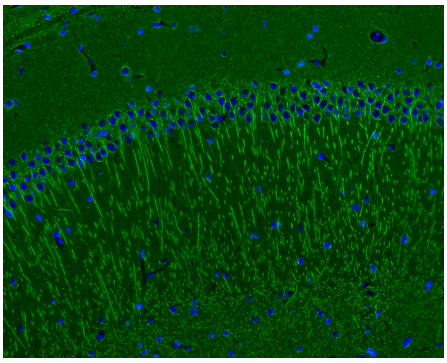

Fig6: Immunofluorescence analysis of frozen mouse hippocampus (CA1) tissue labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (ET1604-17).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1604-17, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

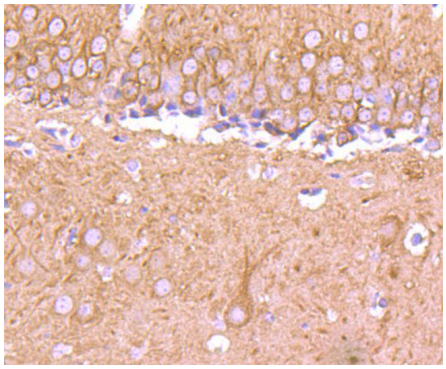

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-Beta III Tubulin antibody.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1604-17, 1/50) for 30 minutes 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.

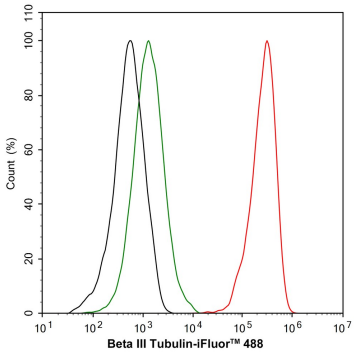

Fig8: Flow cytometric analysis of SH-SY5Y cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-17, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

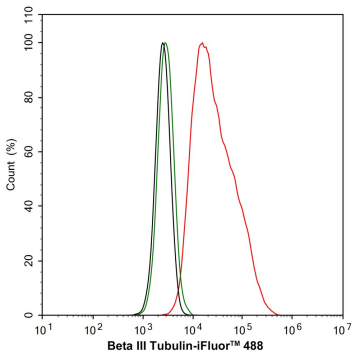

Fig9: Flow cytometric analysis of Neuro-2a cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-17, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

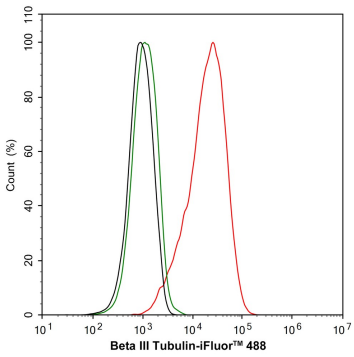

Fig10: Flow cytometric analysis of PC-12 cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-17, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

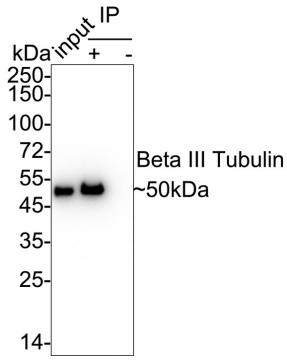

Fig11: Beta III Tubulin was immunoprecipitated from 0.2 mg SH-SY5Y cell lysate with ET1604-17 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1604-17 at 1/10,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: SH-SY5Y cell lysate (input)
Lane 2: ET1604-17 IP in SH-SY5Y cell lysate
Lane 3: Rabbit IgG instead of ET1604-17 in SH-SY5Y cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 2 seconds; ECL: K1801

Background References

1. Long K et al. Integrin signalling regulates the expansion of neuroepithelial progenitors and neurogenesis via Wnt7a and Decorin. Nat Commun 7:10354 (2016).
2. Ren M et al. A biofidelic 3D culture model to study the development of brain cellular systems. Sci Rep 6:24953 (2016).