Anti-beta Tubulin Antibody [SR25-04]

ET1602-4

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	SR25-04

Description: Tubulin is one of several members of a small family of globular proteins. The most common members of the tubulin family are α-tubulin and β-tubulin. The beta-tubulin (relative molecular weight about 50 kDa) is counterpart of alpha-tubulin in tubulin heterodimer, it is coded by multiple tubulin genes and it is also posttranslationally modified. Heterogeneity of subunit is concentrated in C-terminal structural domain. Beta-Tubulin may have bound GTP or GDP. Under certain conditions β-tubulin can hydrolyze its bound GTP to GDP plus Pi, release the Pi, and exchange the GDP for GTP.

Immunogen: Synthetic peptide within Human beta Tubulin aa 308-357 / 444.

Positive control: HeLa cell lysate, 293T cell lysate, MCF7 cell lysate, SH-SY5Y cell lysate, U-2 OS cell lysate, Jurkat cell lysate, Neuro-2a cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, SH-SY5Y, Hela, NIH/3T3, CRC, N2A, PC-12, human fallopian tube tissue, human colon carcinoma tissue, rat kidney tissue, mouse large intestine tissue.

Subcellular location: Cytoplasm

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

Recommended Dilutions:

WB	1:10,000-1:20,000
IF-Cell	1:50-1:200
IHC-P	1:100-1:400
FC	1:500-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

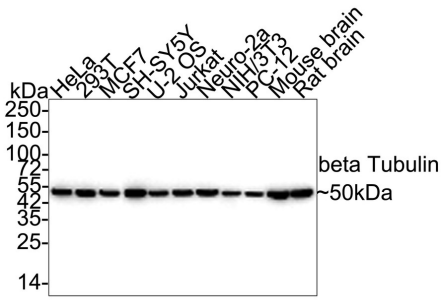

Fig1: Western blot analysis of beta Tubulin on different lysates with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/10,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: 293T cell lysate (15 µg/Lane)
Lane 3: MCF7 cell lysate (15 µg/Lane)
Lane 4: SH-SY5Y cell lysate (15 µg/Lane)
Lane 5: U-2 OS cell lysate (15 µg/Lane)
Lane 6: Jurkat cell lysate (15 µg/Lane)
Lane 7: Neuro-2a cell lysate (15 µg/Lane)
Lane 8: NIH/3T3 cell lysate (15 µg/Lane)
Lane 9: PC-12 cell lysate (15 µg/Lane)
Lane 10: Mouse brain tissue lysate (20 µg/Lane)
Lane 11: Rat brain tissue lysate (20 µg/Lane)

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

Exposure time: 1 minute 20 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 (ET1602-4) at 1/10,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

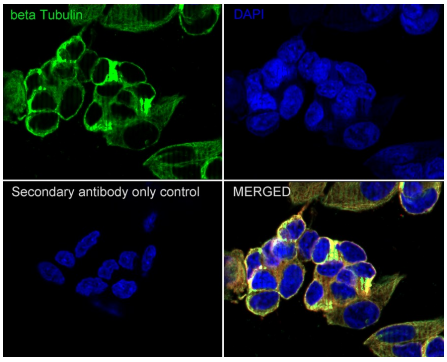

Fig2: Immunocytochemistry analysis of SH-SY5Y cells labeling beta Tubulin with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

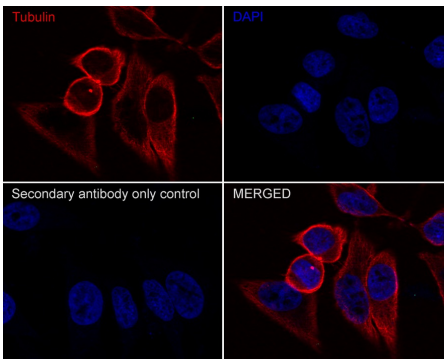

Fig3: Immunocytochemistry analysis of Hela cells labeling beta Tubulin with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) 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.

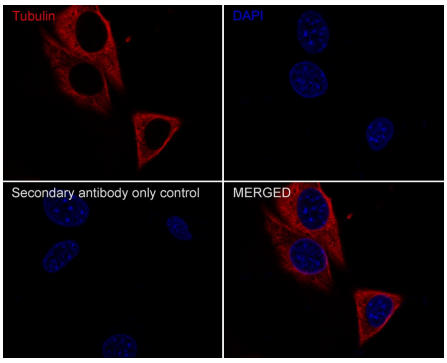

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling beta Tubulin with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) 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.

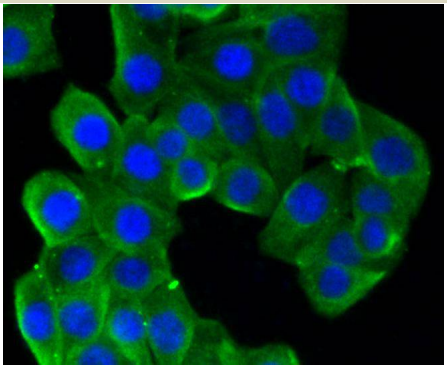

Fig5: ICC staining of beta Tubulin in CRC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1602-4, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

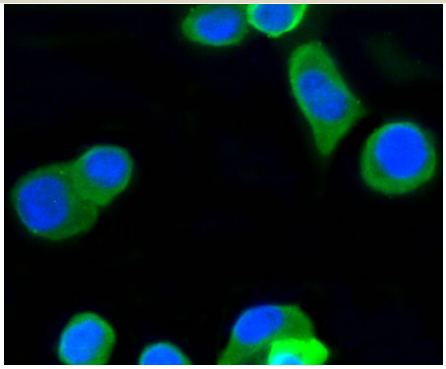

Fig6: ICC staining of beta Tubulin in N2A cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1602-4, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

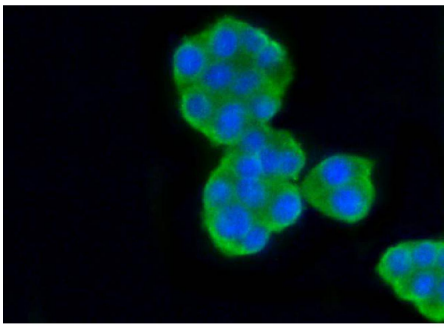

Fig7: ICC staining of beta Tubulin in PC-12 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1602-4, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

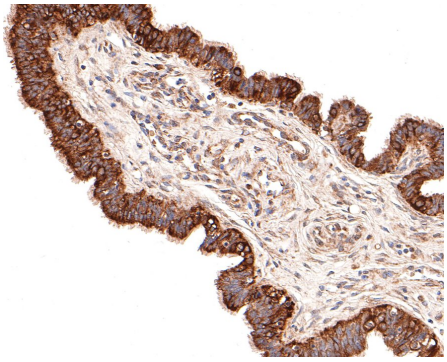

Fig8: Immunohistochemical analysis of paraffin-embedded human fallopian tube tissue with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/100 dilution.

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 (ET1602-4) at 1/100 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.

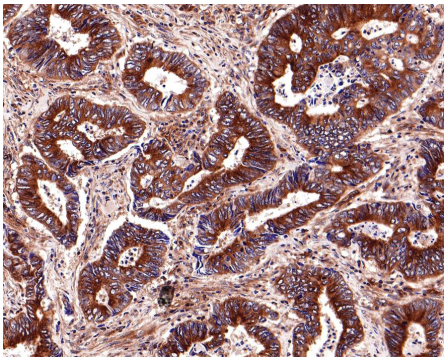

Fig9: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/400 dilution.

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 (ET1602-4) at 1/400 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.

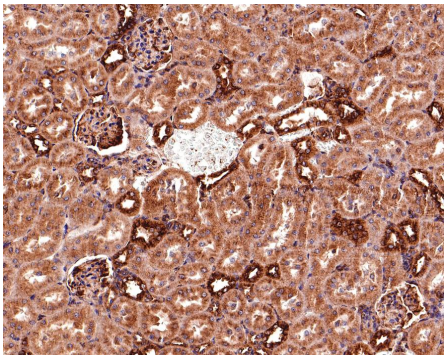

Fig10: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/400 dilution.

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 (ET1602-4) at 1/400 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.

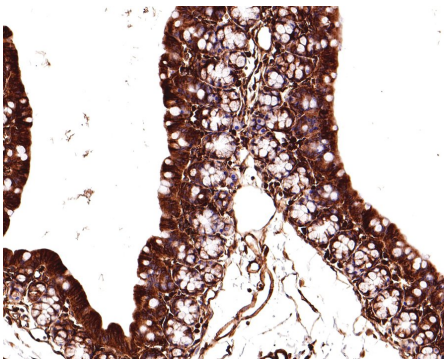

Fig11: Immunohistochemical analysis of paraffin-embedded mouse large intestine tissue with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/400 dilution.

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 (ET1602-4) at 1/400 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.

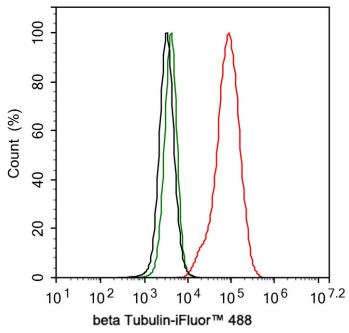

Fig12: Flow cytometric analysis of NIH/3T3 cells labeling beta Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1602-4, 1ug/ml) (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).

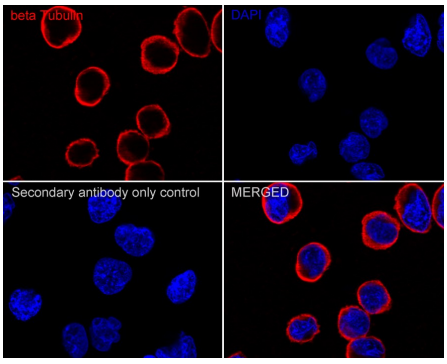

Fig13: Immunocytochemistry analysis of PC-12 cells labeling beta Tubulin with Rabbit anti-beta Tubulin antibody (ET1602-4) at 1/100 dilution.

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 Tubulin antibody (ET1602-4) 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.

Background References

1. "Tumoral and tissue-specific expression of the major human beta-tubulin isotypes."Leandro-Garcia L.J., Leskela S., Landa I., Montero-Conde C., Lopez-Jimenez E., Leton R., .Cytoskeleton 67:214-223(2010).
2. "Five mouse tubulin isotypes and their regulated expression during development."Lewis S.A., Lee M.G.-S., Cowan N.J.J. Cell Biol. 101:852-861(1985).