Anti-Beta III Tubulin Antibody [A8-D10]

M0805-8

Product Type:	Mouse monoclonal IgG2a, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	A8-D10

Description: Tubulin is a compound of subunits of A tubulin and B tubulin. Class III beta tubulin (beta III-tubulin) is a vertebrate tubulin isotype specific to the neurons and mammalian testis cells, making it an ideal neuronal marker. Overexpression of class III beta tubulin is associated with the resistances of microtubule-targeted cancer drugs in lung cancer cell lines, breast cancer cell lines, and ovarian tumors.

Immunogen: Synthetic peptide (KLH-coupled) within human Tubulin beta-3 chain aa 401-450.

Positive control: SH-SY5Y cell lysate, U-87 MG cell lysate, A-172 cell lysate, Neuro-2a cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, HEK-293, SH-SY5Y, PC-12, Neuro-2a, human brain tissue, mouse brain tissue, rat brain tissue, mouse hippocampus tissue, MCF7.

Subcellular location: Cytoplasm. Cytoskeleton. Microtubule.

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

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:500-1:1,000
IHC-P	1:2,000
FC	1:1,000
IF-Tissue	1:200

Storage Buffer: 1*PBS (pH7.4), 0.2% 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: Immunogen affinity purified.

Images

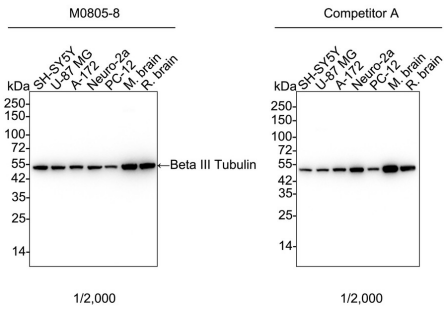

Fig1: Western blot analysis of Beta III Tubulin on different lysates with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: U-87 MG cell lysate
Lane 3: A-172 cell lysate
Lane 4: Neuro-2a cell lysate
Lane 5: PC-12 cell lysate
Lane 6: Mouse brain tissue lysate
Lane 7: Rat brain tissue lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 11 seconds; ECL: K1801;

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 (M0805-8) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% BSA at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

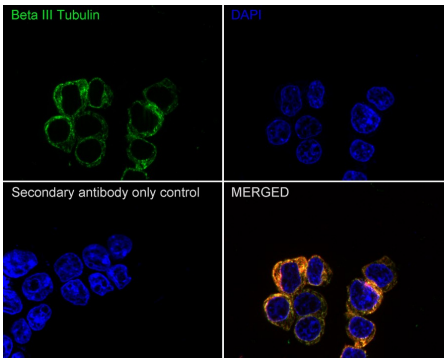

Fig2: Immunocytochemistry analysis of HEK-293 cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

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 III Tubulin antibody (M0805-8) at 1/500 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.

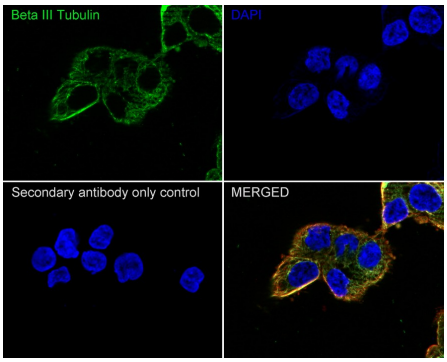

Fig3: Immunocytochemistry analysis of SH-SY5Y cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

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 III Tubulin antibody (M0805-8) at 1/500 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.

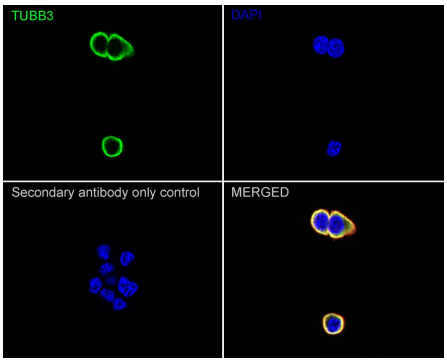

Fig4: Immunocytochemistry analysis of PC-12 cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 III Tubulin antibody (M0805-8) at 1/500 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.

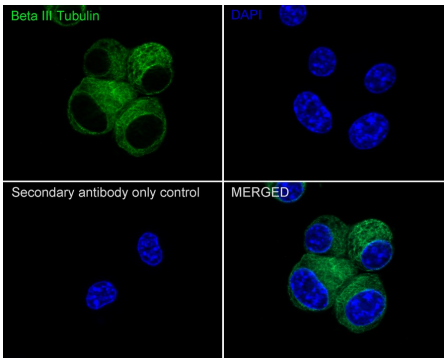

Fig5: Immunocytochemistry analysis of Neuro-2a cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

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 III Tubulin antibody (M0805-8) at 1/500 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.

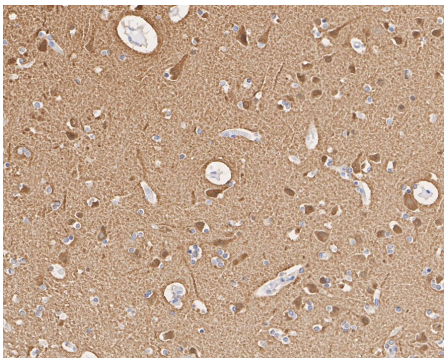

Fig6: Immunohistochemical analysis of paraffin-embedded human brain tissue with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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 (M0805-8) at 1/2,000 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.

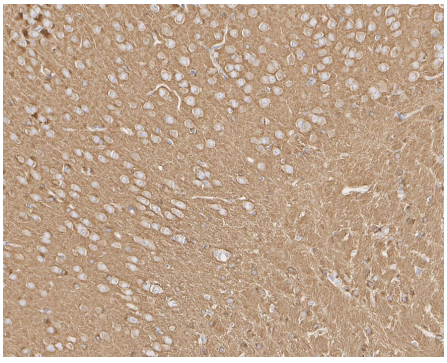

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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 (M0805-8) at 1/2,000 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.

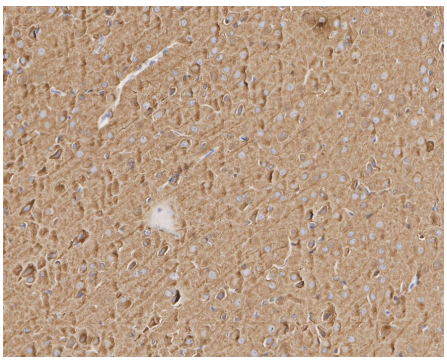

Fig8: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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 (M0805-8) at 1/2,000 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.

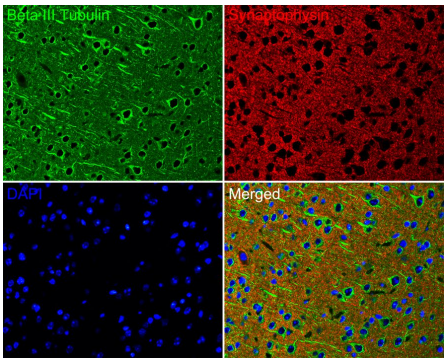

Fig9: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling Beta III Tubulin (M0805-8) and Synaptophysin (ET1606-56).

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 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Beta III Tubulin (M0805-8, green) at 1/200 dilution and Synaptophysin (ET1606-56, red) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Alexa Fluor® 488 conjugate-Goat anti-Mouse IgG and Alexa Fluor® 594 conjugate-Goat anti-Rabbit IgG were used as the secondary antibodies at 1/500 dilution. DAPI was used as nuclear counterstain.

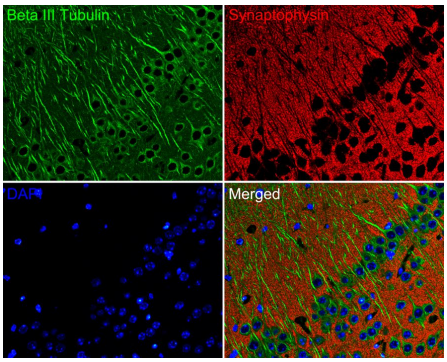

Fig10: Immunofluorescence analysis of paraffin-embedded mouse hippocampus tissue labeling Beta III Tubulin (M0805-8) and Synaptophysin (ET1606-56).

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 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Beta III Tubulin (M0805-8, green) at 1/200 dilution and Synaptophysin (ET1606-56, red) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Alexa Fluor® 488 conjugate-Goat anti-Mouse IgG and Alexa Fluor® 594 conjugate-Goat anti-Rabbit IgG were used as the secondary antibodies at 1/500 dilution. DAPI was used as nuclear counterstain.

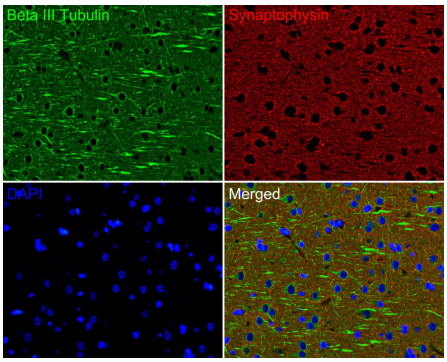

Fig11: Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling Beta III Tubulin (M0805-8) and Synaptophysin (ET1606-56).

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 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Beta III Tubulin (M0805-8, green) at 1/200 dilution and Synaptophysin (ET1606-56, red) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Alexa Fluor® 488 conjugate-Goat anti-Mouse IgG and Alexa Fluor® 594 conjugate-Goat anti-Rabbit IgG were used as the secondary antibodies at 1/500 dilution. DAPI was used as nuclear counterstain.

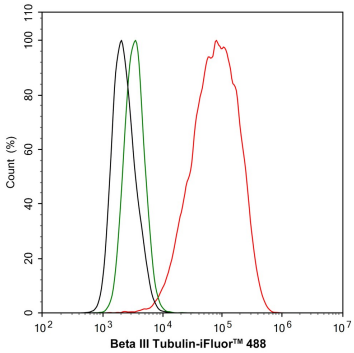

Fig12: Flow cytometric analysis of MCF7 cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (M0805-8, 1/1,000) (red) compared with Mouse IgG1 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Tischfield M A et al. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. Cell 140:74-87 (2010).
2. Fourest-Lieuvin A et al. Microtubule regulation in mitosis: tubulin phosphorylation by the cyclin-dependent kinase Cdk1. Mol Biol Cell 17:1041-1050 (2006).