iFluor™ 488 Conjugated Anti-Beta III Tubulin Antibody [A8-D10]

HA600106F

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

Description: Class III β-tubulin, otherwise known as βIII-tubulin (β3-tubulin) or β-tubulin III, is a microtubule element of the tubulin family. In humans, it is encoded by the TUBB3 gene. It is possible to use monoclonal antibodies and immunohistochemistry to identify neurons in samples of brain tissue, separating neurons from glial cells, which do not express Class III β-tubulin. Class III β-tubulin is one of the seven β-tubulin isotypes identified in the human genome, predominantly in neurons and the testis. It is conditionally expressed in a number of other tissues after exposure to a toxic microenvironment featured by hypoxia and poor nutrient supply. Posttranslational changes including phosphorylation and glycosylation are required for functional activity. Class III β-tubulin's role in neural development has warranted its use as an early biomarker of neural cell differentiation from multi potent progenitors. TUBB3 inactivation impairs neural progenitor proliferation. Rescue experiments demonstrate the non-interchangeability of TUBB3 with other classes of β-tubulins which cannot restore the phenotype resulting from TUBB3 inactivation. Congenital neurologic syndromes associated with TUBB3 missense mutations demonstrate the critical importance of class III β-tubulin for normal neural development. 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.

Conjugate:	iFluor™ 488, Ex: 491nm; Em: 516nm.
Immunogen:	Synthetic peptide (KLH-coupled) within human Tubulin beta-3 chain aa 401-450.
Positive control:	HepG2, SH-SY5Y, rat brain tissue, mouse brain tissue.
Subcellular location:	Cytoplasm. Cytoskeleton. Microtubule.
Database links:	SwissProt: Q13509 Human | Q9ERD7 Mouse | Q4QRB4 Rat
Recommended Dilutions:	
IF-Cell	1:50
FC	1:500-1:1,000
IF-Tissue	1:200
Storage Buffer:	Preservative: 0.02% Sodium azide Constituents: 30% Glycerol, 1% BSA, 68.98% PBS.
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

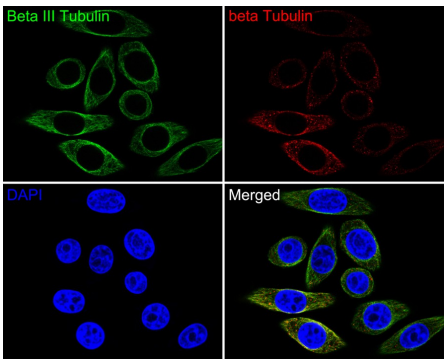

Fig1: Immunocytochemistry analysis of HepG2 cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (HA600106F) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, 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 Mouse anti-Beta III Tubulin antibody (HA600106F, iFluor™ 488) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. 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™ 647, HA1123) were used as the secondary antibody at 1/1,000 dilution.

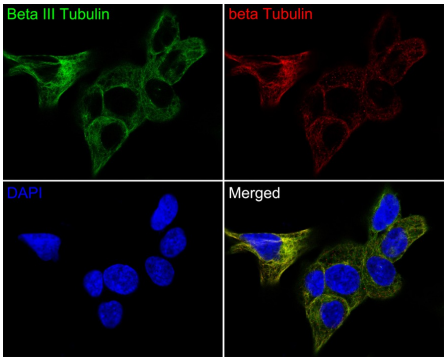

Fig2: Immunocytochemistry analysis of SH-SY5Y cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (HA600106F) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, 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 Mouse anti-Beta III Tubulin antibody (HA600106F, iFluor™ 488) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. 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™ 647, HA1123) were used as the secondary antibody at 1/1,000 dilution.

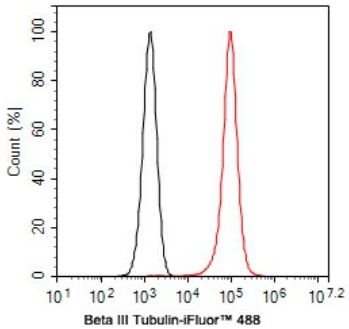

Fig3: Flow cytometric analysis of SH-SY5Y cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then incubated for 1 hour at +4℃ with Beta III Tubulin (HA600106F, red, 1ug/ml). Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

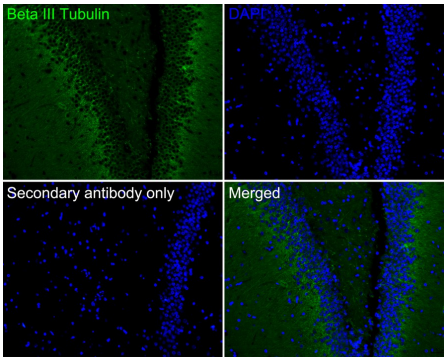

Fig4: Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (HA600106F) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA600106F, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Nuclei were counterstained with DAPI (blue).

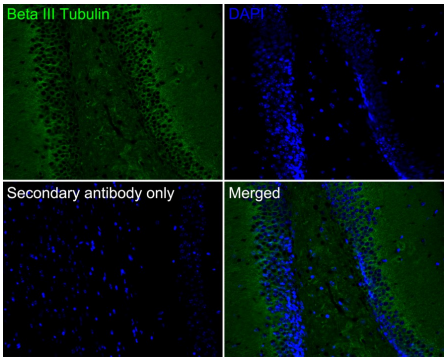

Fig5: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (HA600106F) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA600106F, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Nuclei were counterstained with DAPI (blue).

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).