# Anti-Beta III Tubulin Antibody [A8-D10]

## M0805-8

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|---|---|
| **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, 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:** Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

**Purity:** Immunogen affinity purified.

## Images

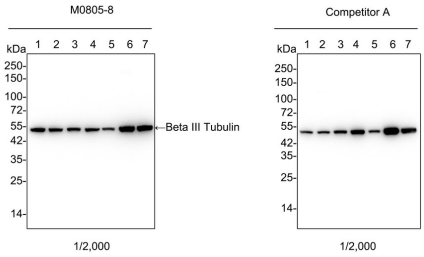


**Fig1:** Western blot analysis of Beta III Tubulin on different lysates with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

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;

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.

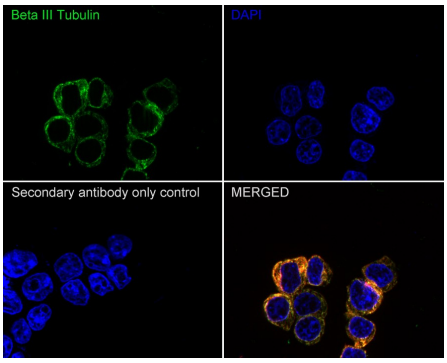


**Fig2:** Immunocytochemistry analysis of HEK-293 cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

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.

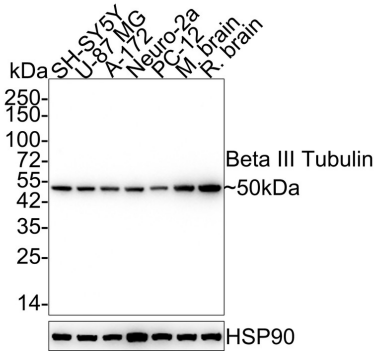


**Fig3:** Western blot analysis of Beta III Tubulin on different lysates with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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;

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 was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

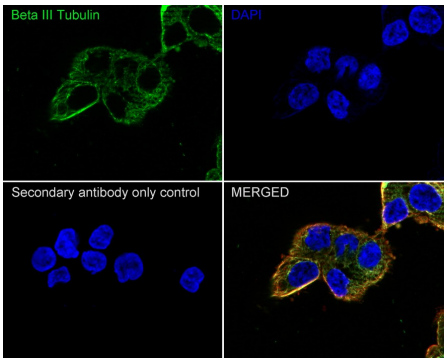


**Fig4:** Immunocytochemistry analysis of SH-SY5Y cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

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.

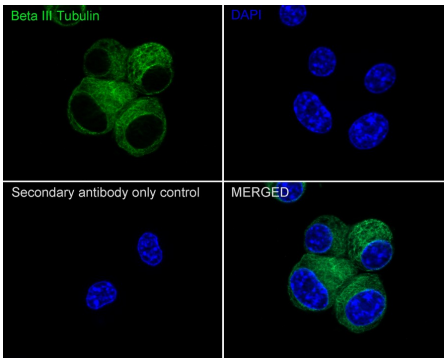


**Fig5:** Immunocytochemistry analysis of Neuro-2a cells labeling Beta III Tubulin with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/500 dilution.

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.

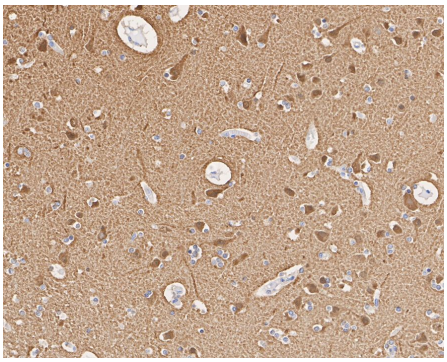

**Fig6:** Immunohistochemical analysis of paraffin-embedded human brain tissue with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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.

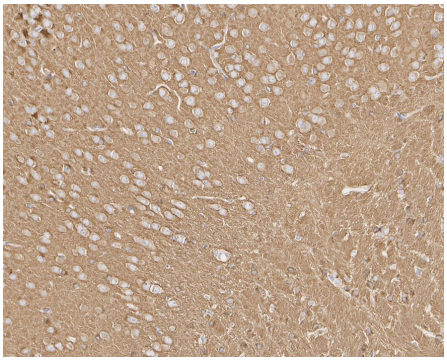

**Fig7:** Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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.

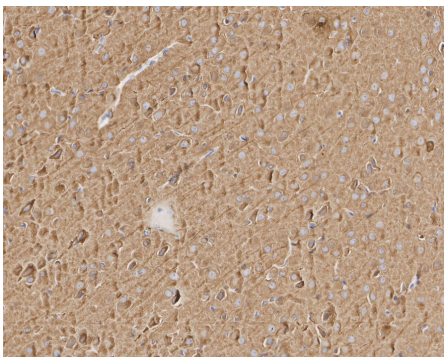

**Fig8:** Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

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.

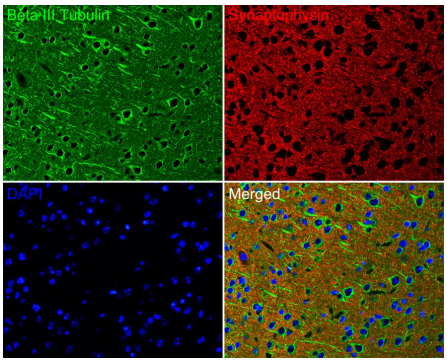


**Fig9:** Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling Beta III Tubulin (M0805-8) and Synaptophysin (ET1606-56).

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.

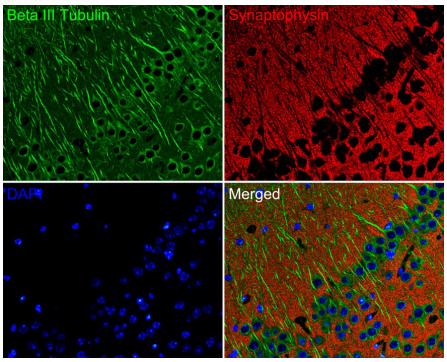


**Fig10:** Immunofluorescence analysis of paraffin-embedded mouse hippocampus tissue labeling Beta III Tubulin (M0805-8) and Synaptophysin (ET1606-56).

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.

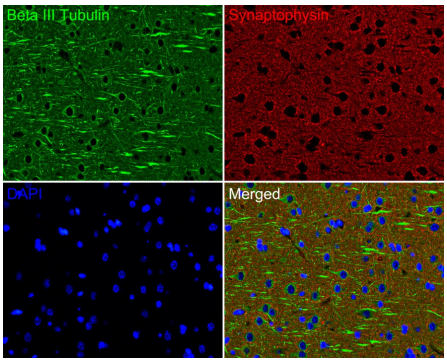


**Fig11:** Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling Beta III Tubulin (M0805-8) and Synaptophysin (ET1606-56).

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.

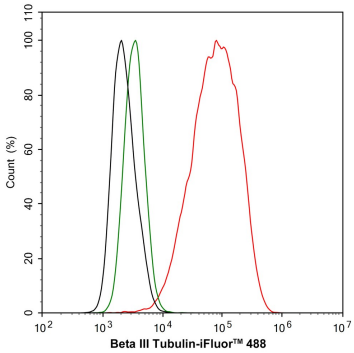


**Fig12:** Flow cytometric analysis of MCF7 cells labeling Beta III Tubulin.

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).