Anti-beta Tubulin Antibody [A1-A4] M1305-2

Product Type:	Mouse monoclonal IgG3, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	A1-A4
Description:	Tubulins is one of several members of a small family of globular proteins. The most common members of the tubulins family are α -tubulins and β -tubulins. The beta-tubulins (relative molecular weight about 50 kDa) is counterpart of alpha-tubulin in tubulins heterodimer, it is coded by multiple tubulins genes and it is also posttranslationally modified. Heterogeneity of subunit is concentrated in C-terminal structural domain. Beta-Tubulins 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 151-200 / 444.
Positive control:	Hela cell lysates, NIH/3T3 cell lysates, PC-12 cell lysates, SKOV-3 cells, HeLa, 293, human tonsil tissue, human spleen tissue, human colon tissue, rat brain tissue, NIH/3T3.
Subcellular location:	Cytoplasm, Cytoskeleton, Microtubule.
Database links:	SwissProt: P07437 Human P99024 Mouse P69897 Rat
Recommended Dilutions: WB IF-Cell IHC-P	1:5,000-1:20,000 1:100-1:500 1:50-1:200
FC	1:100-1:1,000
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4 $^\circ\!\!\mathbb{C}$. Store at +4 $^\circ\!\!\mathbb{C}$ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20 $^\circ\!\!\mathbb{C}$ long term.
Purity:	Protein A affinity purified.

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47.

Images

Fig1: Western blot analysis of beta Tubulin on Hela cell lysates with Mouse anti-beta Tubulin antibody (M1305-2).

Hela cell lysates at 10 µg/Lane.

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

8% 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 (M1305-2) at serial dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of beta Tubulin on NIH/3T3 cell lysates with Mouse anti-beta Tubulin antibody (M1305-2).

NIH/3T3 cell lysates at 10 µg/Lane.

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

8% 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 (M1305-2) at serial dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

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150-100-75-50-37-Beta tubulin ~50 kDa

1/4,000 /8,000 /16,000 /32,000

/1,000

kDa

250





Fig3: Western blot analysis of beta Tubulin on PC-12 cell lysates with Mouse anti-beta Tubulin antibody (M1305-2).

PC-12 cell lysates at 10 µg/Lane.

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

8% 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 (M1305-2) at serial dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

Fig4: Western blot analysis of beta Tubulin on different lysates with Mouse anti-beta Tubulin antibody (M1305-2) at 1/1,000 dilution.

Lane 1: HeLa cell lysate Lane 2: HepG2 cell lysate Lane 3: NIH/3T3 cell lysate Lane 4: PC-12 cell lysate Lane 5: C6 cell lysate Lane 6: Vero cell lysate

Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 52 kDa Observed band size: 52 kDa

Exposure time: 2 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 (M1305-2) at 1/1,000 dilution was used in 5% NFDM/TBST at 4° C overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.



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Fig5: Immunocytochemistry analysis of HeLa cells labeling beta Tubulin with Mouse anti-beta Tubulin antibody (M1305-2) at 1/100 dilution.

Cells were fixed in 100% precooled methanol 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 Tubulin antibody (M1305-2) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor $^{\circ}$ 594, HA1126) 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: Immunocytochemistry analysis of 293 cells labeling beta Tubulin with Mouse anti-beta Tubulin antibody (M1305-2) 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 Mouse anti-beta Tubulin antibody (M1305-2) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluorTM 594, HA1126) 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.



Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-beta tubulin antibody. Counter stained with hematoxylin.

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Fig8: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-beta tubulin antibody. Counter stained with hematoxylin.



Fig9: Immunohistochemical analysis of paraffin-embedded human colon tissue using anti-beta tubulin antibody. Counter stained with hematoxylin.



Fig10: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-beta tubulin antibody. Counter stained with hematoxylin.



Fig11: Flow cytometric analysis of HeLa cells labeling beta Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (M1305-2, 1µg/mL) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Cells were fixed and permeabilized. Then stained with the primary antibody (M1305-2, 1µg/mL) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 °C. 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 Mouse anti-beta Tubulin antibody (M1305-2) 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 Mouse anti-beta Tubulin antibody (M1305-2) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluorTM 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 $+4^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 1594, HA1122) were used as the secondary antibody at 1/1,000 dilution.



Fig14: Flow cytometric analysis of PC-12 HeLa cells labeling beta Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (M1305-2, 1µg/mL) (red) compared with Mouse IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

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)
- "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)

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