

Product Information

Anti- β -Tubulin III

Developed in Rabbit
Affinity Isolated Antibody

Product Number **T 2200**

Product Description

Anti- β -Tubulin III is developed in rabbit using as immunogen a synthetic peptide corresponding to amino acid residues 441-450 of human β -tubulin III (Ala⁴⁴⁶ to Ser⁴⁴⁶ substitution) with N-terminal added cysteine, conjugated to KLH. The sequence is conserved in mammals. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti- β -Tubulin III recognizes human, mouse, and rat β -tubulin III. Applications include immunoblotting (~55 kDa), immunoprecipitation, and indirect immunofluorescence staining of cultured cells and frozen sections. Additional weak bands may be detected when immunoblotting some extract preparations. Detection of β -tubulin III by immunoblotting is specifically inhibited with the immunizing peptide. When using the antibody in immunoblotting, no β -tubulin III is detectable in human white blood cells or platelets.

α/β -Tubulin, an integral component of microtubules, is present in almost all eukaryotic cells. Microtubules function as structural and mobile elements in mitosis, intracellular transport, flagellar movement, cell morphogenesis, and other cytoskeletal functions.¹ α/β -Tubulin occurs mostly as soluble (approx. 100-110 kDa) heterodimeric sets of α - and β -tubulin isotypes or as polymers in assembled microtubules.² Within either set of polypeptides, individual subunits diverge from each other (both within and across species) at less than 10% of the amino acid positions.³ The most extreme diversity is localized to the 15 residues of the carboxy-terminal.

Research has been centered on the hypothesis that the β -tubulin isotypes contribute to unique functional properties. It has been reported that the different isotypes

of tubulin differ from each other in their ability to polymerize into microtubules.⁴ α/β -Tubulin heterogeneity is further increased by numerous post-translational covalent modifications.^{6,7} The most complex pattern of isotype distribution in tissues is seen in the vertebrate β -tubulins.^{7,8} Six evolutionarily conserved isotypes of β -tubulin have been identified (designated β I- β VI). In mammals and birds, β -tubulin I is constitutive and is found in most tissues. β -Tubulin II is found in many tissues, but mainly in the brain. The synthesis of β -tubulin II increases in regeneration and development of neurons.

β -Tubulin III (also designated β -4 chain) is found in the brain and dorsal root ganglia and appears to be localized to neurons of the central and peripheral nervous system, where its expression seems to increase during axonal outgrowth. β -Tubulin III is reported to be enriched in neuronal mitochondria.⁹ Although considered a highly specific marker for neurons, β -tubulin III is also found in Sertoli cells of the testis, spermatozoa tails, certain lung cells, and apparently some breast stromal cells.^{10,11} β -tubulin III is also found in certain tumors of non-neural origin, such as lymphoma, squamous cell carcinoma, giant cell glioblastoma multiforme, oligodendroglioma, and malignant melanoma but not in their non-neoplastic adult counterparts. Its expression is increased in various chemoresistant epithelial cell lines and gliomas and lung cancer with ascending grade of malignancy. This isotype has been identified as a target for auto-antibodies formed in neuroblastoma patients.¹² Unlike other β isotypes, β -tubulin III contains a phosphorylatable serine at position 444.^{13,14}

β -Tubulin IV occurs in mammals as two subtypes. β -Tubulin IVa is brain specific, whereas β -tubulin IVb is

ubiquitous, and both appear to be constitutive. In chickens, there is only one form of β -IV, which is expressed at low levels in many tissues, but is the major β isotype in the testis. β -Tubulin V in chickens is apparently ubiquitous outside of the brain, and is also expressed in a variety of cultured mammalian cells. β -Tubulin VI, the most evolutionary divergent isotype, is apparently restricted to hematopoietic tissues and expressed in chicken erythrocytes and mammalian platelets, spleen, bone marrow, and other blood-forming tissues.

Reagent

Anti- β -Tubulin III is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Antibody Concentration: Approx. 0.6 mg/ml

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

By immunoblotting, a working antibody concentration of 0.2-0.4 μ g/ml is recommended using whole extracts of mouse brain or cultured human neuroblastoma SH-SY5Y, and a chemiluminescent reagent.

By immunoprecipitation, 10 μ g of the antibody will immunoprecipitates β -tubulin III from 250 μ g RIPA extract of cultured rat pheochromocytoma PC12 cells.

By indirect immunofluorescence, a working antibody concentration of 10-20 μ g/ml is recommended using rat pheochromocytoma PC12 cells.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working concentrations by titration.

References

1. Oakley, B.R., Trends Cell Biol., **2**, 1-5 (1992).
2. Sullivan, K.F., et al., Annu. Rev. Cell Biol., **4**, 687-716 (1988).
3. Joshi, H.C. and Cleveland, D.W., Cell Motil. Cytoskel., **16**, 159-163 (1990).
4. Banerjee, A., et al., J. Biol. Chem., **265**, 1794-1799 (1990).
5. MacRae, T.H., Eur. J. Biochem., **244**, 265-278 (1997).
6. Roach, M.C., et al., Cell Motil. Cytoskel., **39**, 273-285 (1998).
7. Luduena, R.F., Int. Rev. Cytol., **178**, 207-275 (1998).
8. Luduena, R.F., Mol. Biol. Cell, **4**, 445-457 (1993).
9. Carre, M., et al., J. Biol. Chem., **277**, 33664-33669 (2002).
10. Draberova, E., et al., Histochem. Cell Biol., **109**, 231-239 (1998).
11. Dozier, J.H., et al., Breast Cancer Res., **5**, R157-169 (2003).
12. Prasanna, L., Clin. Cancer Res., **6**, 3949-3956 (2000).
13. Luduena, R.F., et al., FEBS Lett., **230**, 142-146 (1988).
14. Khan, I.A., and Luduena, R.F., Biochemistry, **35**, 3704-3711 (1996).

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