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Purified Mouse anti-Alpha-Tubulin Monoclonal Antibody

CLX135AP

Lot:

Size: 0.1 mg

Clone: TU-01

Isotype: Mouse IgG1

Specificity: The antibody TU-01 recognizes the defined epitope (aa 65-97) on N-terminal structural domain of alpha-tubulin.

Immunogen: Fraction of tubulin purified from porcine brain by two cycles of polymerization - depolymerization.

Species Reactivity: Broad species reactivity

Application: **Western Blotting**
Recommended dilution: 1-2 µg/ml, incubation 60 min in room temperature
Positive control: HPB-ALL human peripheral blood leukemia cell line (incubation 60 min)
Porcine brain lysate (incubation 90 min)
Sample preparation: Resuspend approx. 50 mil. cells in 1 ml cold Lysis buffer (1% laurylmaltoside in 20 mM Tris/Cl, 100 mM NaCl pH 8.2, 50 mM NaF including Protease inhibitor Cocktail). Incubate 60 min on ice. Centrifuge to remove cell debris. Mix lysate with reducing Laemmli SDS-PAGE sample buffer.
Application note: Reducing conditions.

Immunohistochemistry (paraffin sections)

Recommended dilution: 5 µg/ml
Positive tissue: heart

Immunocytochemistry

Staining technique: fixed and permeabilized cells

Purity: > 95% (by SDS-PAGE)

Purification: Purified from ascites by precipitation methods.

Concentration: 1 mg/ml

Storage Buffer: Phosphate buffered saline (PBS) with 15 mM sodium azide, approx. pH 7.4

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Storage / Stability: Store at 2-8°C. Do not use after expiration date stamped on vial label. For long-term storage aliquot and store at -20°C. Avoid freeze/thaw cycles.

Background: The microtubules are intracellular dynamic polymers made up of evolutionarily conserved polymorphic alpha/beta-tubulin heterodimers and a large number of microtubule-associated proteins (MAPs). The microtubules consist of 13 protofilaments and have an outer diameter 25 nm. Microtubules have their intrinsic polarity; highly dynamic plus ends and less dynamic minus ends. Microtubules are required for vital processes in eukaryotic cells including mitosis, meiosis, maintenance of cell shape and intracellular transport. Microtubules are also necessary for movement of cells by means of flagella and cilia. In mammalian tissue culture cells microtubules have their minus ends anchored in microtubule organizing centers (MTOCs). The GTP (guanosinotriphosphate) molecule is an essential for tubulin heterodimer to associate with other heterodimers to form microtubule. In vivo, microtubule dynamics vary considerably. Microtubule polymerization is reversible and a populations of microtubules in cells are on their minus ends either growing or shortening – this phenomenon is called dynamic instability of microtubules. On a practical level, microtubules can easily be stabilized by the addition of non-hydrolysable analogues of GTP (eg. GMPPCP) or more commonly by anti-cancer drugs such as Taxol. Taxol stabilizes microtubules at room temperature for many hours. Using limited proteolysis by enzymes both tubulin subunits can be divided into N-terminal and C-terminal structural domains. The alpha-tubulin (relative molecular weight around 50 kDa) is globular protein that exists in cells as part of soluble alpha/beta-tubulin dimer or it is polymerized into microtubules. In different species it is coded by multiple tubulin genes that form tubulin classes (in human 6 genes). Expressed tubulin genes are named tubulin isotypes. Some of the tubulin isotypes are expressed ubiquitously, while some have more restricted tissue expression. Alpha-tubulin is also subject of numerous post-translational modifications. Tubulin isotypes and their posttranslational modifications are responsible for multiple tubulin charge variants - tubulin isoforms. Heterogeneity of alpha-tubulin is concentrated in C-terminal structural domain.

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