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ProductInformation

Anti- g-Tubulin
Developed in Rabbit
IgG Fraction of Antiserum

Product No. **T3559**

Product Description

Anti-γ-Tubulin is developed in rabbits using a synthetic peptide EEFATEGTDRKDVFFY-K corresponding to the N-terminal region of human γ-tubulin (amino acids 38-53, with C-terminally added lysine), conjugated to KLH as immunogen. This sequence is identical in mouse γ-tubulin and highly conserved among species (*Drosophila, Aspergillus* and yeast γ-tubulin), and is not found in other tubulin isoforms (α and β tubulins).

Anti-γ-Tubulin recognizes an epitope located in the N-terminal region of γ-tubulin (amino acids 38-53). The product may be applied in immunoblotting of A431 cultured cells whole extract (γ-tubulin, 48 kDa), and in immunocytochemical staining of cultured chicken fibroblast cells. Staining of γ-tubulin (48 kDa) by immunoblotting is specifically inhibited with γ-tubulin peptide (human, amino acids 38-53 with C-terminally added lysine).

γ-Tubulin (48kDa) is a ubiquitous and highly conserved protein within the microtubule organizing centers (MTOCs) in eukaryotic cells.[1] It is related to α- and β-tubulin and is a member of the tubulin superfamily of proteins. Many cellular functions are dependent on the proper organization of microtubules, since they are essential for mitosis, meiosis, some forms of organellar movement, and other cytoskeletal functions[1] Temporal and spatial regulation of microtubule assembly is critical for the correct assembly of the mitotic apparatus and of the cytoplasmic microtubule array. Microtubules are composed primarily of two similar proteins, α- and β-tubulin, which form a heterodimer that assembles into microtubules. The properties of microtubules are due in part to other microtubule-associated proteins which coassemble with α- and β-tubulin, and alter the assembly characteristics of microtubules. A special class of microtubule-associated proteins (dynein, kinesin and related proteins) is involved in microtubule-based motility, while other proteins are involved in the attachment of microtubules to kinetochores and promote the assembly of microtubules at the MTOCs, such as the centrosome.[2,3] Centrosomes nucleate the assembly of microtubules and establish the polarity of microtubules, with the minus end centrosome proximal. γ-Tubulin binds microtubule minus ends and is responsible for mediating the link between microtubules and the centrosome.[1,4] It functions as the microtubule nucleator at the MTOC. It binds to the β-tubulin half of the tubulin molecule, thus establishing the polarity of a microtubule, leaving the α-tubulin half exposed at the plus end. γ-Tubulin abundance is less than 1% of the level of either α- or β-tubulin.[5] Moreover, unlike α- and β-tubulin, it is not a component of microtubules, being localized at the MTOC.[1,6-8] γ-Tubulin shares approximately 28-32% identity with α-tubulin from various organisms and 32-36% identity with β-tubulins. Some regions (including regions thought to be involved in GTP binding) are highly conserved among α-, β- and γ-tubulins. The detection, localization and characterization of proteins involved in microtubule function is fundamental to the understanding of mitosis, meiosis and the microtubule cytoskeleton. Antibodies reacting specifically with γ-tubulin[5-8] serve as an essential tool in the detection of the presence and role of this molecule in various cellular settings.

Reagents

The product is provided as IgG fraction with 15mM sodium azide as a preservative.

Precautions

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 hazardous and safe handling practices.

Product Profile
A minimum working dilution of 1:1,000 is determined by immunoblotting using A431 cultured cells whole extract.

A minimum working dilution of 1:5,000 is determined by immunofluorescent staining of centrosome associated dots containing γ tubulin, in methanol/acetone fixed chicken fibroblasts.
In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Storage
For continuous use, store at 2-8°C for a maximum of one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Oakley, B.R., Trends Cell Biol., **2,** 1 (1992).
2. Stebbings, H., Nature, **336**, 14 (1988).
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4. Oakley, C.E., and Oakley, B.R., Nature, **338**, 662 (1989).
5. Stearns, T., et al., Cell, **65**, 825 (1991).
6. Zheng, Y., et al., Cell, **65**, 817 (1991).
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8. Joshi, H.C., et al., Nature, **356**, 80 (1992).

Immunoblotting Procedure of Whole Cell Extract
Preparation of Whole Cell Culture Extracts
1. Grow A431 cells to confluence in 10cm plates containing 10% FCS in DMEM.
2. Remove medium from culture dishes
3. Rinse plates with ice cold PBS pH 7.4 (2 x 10 ml).
4. Scrape cells and add 0.5 ml/plate of (1x) sample buffer.
5. Boil sample for 5 min. at 95ºC.
6. Aliquot sample of cells extract and store at -70ºC.

Immunoblotting Reagents and Equipment
1. A431 whole cells extract (freshly prepared).
2. 10% polyacrylamide slab minigel with 5% stacking gel (80 x 80 x 1.5 mm).
3. Nitrocellulose membrane (0.45µm).
4. Prestained HMW markers (Product C3312).
5. Blocking Buffer: 10% dry milk (w/v) in 10 mM phosphate buffered saline (PBS), pH 7.4.
6. Dilution Buffer: 1% BSA in PBS, pH 7.4, containing 0.05% Tween-20.
7. Washing Buffer: PBS, pH 7.4, containing 0.05% Tween-20.
8. Tubulin peptide (human, amino acids 38-53 with C-terminally added lysine). Dissolve in deionized H_2O at 0.5 mg/ml. Store aliquots at -20ºC.
9. Primary antibody: Anti-K Tubulin at appropriate dilution in dilution buffer.
10. Secondary Antibody: Alkaline phosphatase Anti-rabbit IgG (Gt) (Product A9919) at appropriate dilution in dilution buffer.
11. Substrate: BCIP/NBT Tablets (Product B5655).
12. Electrophoresis and transfer apparatus.

Immunoblotting Procedure
Note: In order to obtain best results in different preparations it is recommended to optimize procedure conditions (antibody dilutions, incubation times, blocking conditions etc.), for a specific application.
1. Resolve whole cell extracts (250µl/slab) on precast 10% polyacrylamide minigel.
2. Run SDS-PAGE at room temperature.
3. Perform transfer for 1 hour at room temperature to nitrocellulose membrane.
4. Block nitrocellulose membrane in blocking buffer for at least 1 hour at room temperature.
5. Incubate membrane with primary antibody dilutions for 2 hours at room temperature [(a)].
6. Wash membrane with washing buffer 4x5 min.
7. Incubate membrane with secondary antibody at recommended dilution in dilution buffer for 1 hour at room temperature.
8. Wash membrane with washing buffer 4 x 5 min. Wash 1 x 5 min. in deionized water.
9. Prepare substrate and incubate membrane with substrate solution.
10. Wash membrane thoroughly with deionized water.
11. Air-dry blots on filter paper.

[(a)]Note: For specific inhibition of K tubulin band (48 kDa band) it is recommended to incubate prediluted antibody with K tubulin peptide (38-53) , 10 µg/ml (final concentration), for 2 hours at room temperature or overnight at 4ºC.

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