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ProductInformation

Anti-γ-Tubulin Cy3 Conjugate
Developed in Rabbit
Affinity Isolated Antibody

Product Number **C 7604**

Product Description

Anti-γ-Tubulin is developed in rabbit using a synthetic peptide 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 specific for γ-tubulin and not found in other members of the tubulin family such as α,β,δ and ε tubulins. This sequence is identical in mouse and rat. γ-Tubulin and highly conserved among species (*Drosophila*, *Aspergillus*, and yeast γ-tubulin). Anti-γ-tubulin is affinity-purified using the immunogenic peptide immobilized on agarose. The product is prepared by conjugation of Cy3[1] to affinity purified anti-γ-tubulin. The conjugate is purified by gel filtration to remove unbound Cy3 fluorophore.

Anti-γ-Tubulin, Cy3 Conjugate recognizes human and chicken γ-tubulin. The conjugate may be used for direct immunofluorescence (immunocytochemistry) of methanol/acetone fixed cells. Staining of γ-tubulin in immunocytochemistry is specifically inhibited with γ-tubulin immunizing peptide (human, amino acids 38-53, with C-terminally added lysine).

γ-Tubulin (48 kDa) is a widely expressed and highly conserved protein within the microtubule organizing centers (MTOCs) or centrosome in eukaryotic cells.[2] It is a member of the tubulin superfamily of proteins, which include α- and β-tubulin and the newly discovered centrosomal-associated proteins, δ- and ε-tubulin.[2, 3] The microtubule cytoskeleton consists of a dynamic, highly polarized network of microtubules filaments, microtubule-associated proteins, microtubule motors and microtubule-organizing proteins. The proper organization of microtubules is essential for cell division and chromosome segregation, directed cell movement, interphase cytoplasmic organization and other cytoskeletal functions.[2] Microtubules are complex polymers of α-tubulin/β-tubulin heterodimers.

Centrosomes nucleate the assembly of microtubules and establish the polarity of microtubules. γ-tubulin has an essential role in microtubule nucleation by the centrosomes.[4-10] γ-Tubulin does not polymerize with α-tubulin/β-tubulin, but instead it is localized to the centrosome and to the cytoplasm.[2, 5-7] γ-Tubulin is found as part of a large protein complex containing at least five other proteins, and has a shape of a ring (γ-tubulin ring complex, γ-TuRC) that is roughly the same diameter as a microtubule.[10-14] γ-Tubulin binds the microtubule minus ends and is responsible for mediating the link between microtubules and the centrosome.[2, 7] 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.[6] γ-Tubulin shares approximately 28-32% identity with α-tubulin from various organisms, 32-36% identity with β-tubulins and 29-30% identity with δ- and ε-tubulin, respectively. Some regions (including regions thought to be involved in GTP binding) are highly conserved among α-, β- and γ-, δ- and ε-tubulins.[3]

Reagent

Anti-γ-Tubulin, Cy3 Conjugate 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. 0.5 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety 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
Store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedure
Immunofluorescence Labeling of Cultured Cells

Materials
1. Coverslips
2. Cells (e.g., human or chicken fibroblasts) in DMEM medium (Product No. D 5546) with 10% fetal calf serum (Product No. F 2442)
3. 10 mM phosphate buffered saline (PBS) pH 7.2 to 7.4 (Product No. P 4417)
4. Diluent: PBS containing 1% BSA (Product No. P 3688)
5. Absolute methanol, cooled to −20 °C
6. Acetone, Analytical grade, cooled to −20 °C
7. Aqueous mounting media
8. Cy3 Conjugated Rabbit Anti-γ-Tubulin (Product No. C 7604)

Cell Growth and Fixation
1. Collect cells from tissue culture dish at a stage of almost confluency, wash with medium and seed onto coverslips. Seed 1 x 10^4 to 2 x 10^4 cells per coverslip and grow cells in incubator for 1 to 2 days or until semi-confluent. Do not change medium. Do not grow cell to confluence.
2. Remove coverslips from incubator, aspirate medium.
3. Wash twice with PBS and remove solution by aspiration.
4. Add enough cold methanol to cover the cell layer. Incubate 10 minutes at −20 °C. Aspirate solution.
5. Rinse cell layer **once** for **1 minute** with cold acetone, and aspirate.
6. Wash 2x with PBS containing 1% BSA. Rehydrate in PBS containing 1% BSA for at least 30 minutes prior to labeling with antibody.

Direct Immunofluorescence Labeling
1. Dilute Cy3 Conjugated Rabbit Anti-γ-Tubulin in PBS containing 1% BSA. Add enough diluted antibody to cover the cell layer and incubate coverslips for 60 minutes at room temperature.
2. Wash 3x with PBS containing 1% BSA, at least 5 minutes each.
3. Drain excess solution by touching edge of coverslips on paper toweling.
4. Invert coverslips onto mounting media applied on glass slides.
5. Observe under UV/visible fluorescent microscope at 568 nm. Mounted preparations can be stored in the dark at 2-8 °C.

Notes
1. Do not allow cell layer to dry out at any time during the aforementioned procedure.
2. In case of excessive background staining, remove aggregates from the labeled reagent by centrifuging for 15 minutes immediately prior to use.

Product Profile
A minimum working dilution of 1:200 is determined by direct immunofuorescent staining of methanol/acetone-fixed chicken fibroblast cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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