Product Information

# Anti-β-Tubulin–FITC Antibody, Mouse Monoclonal

Clone TUB 2.1, purified from hybridoma cell culture

**F2043**

## Product Description

Monoclonal Anti-β-Tubulin (mouse IgG1 isotype) is derived from the hybridoma TUB 2.1 produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with purified rat brain tubulin.[1] The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. No. ISO2. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) isomer I to Protein A purified monoclonal anti-β-Tubulin antibody. The conjugate is purified by gel filtration to remove unbound FITC fluorophore.

Monoclonal Anti-β-Tubulin reacts with the chymotryptic β-Lc and β-Sc tubulin fragments and does not exhibit any apparent mouse brain β-tubulin isoform related specificity.[2,3] The antibody epitope resides between amino acids 281-446 in the carboxy-terminal part of all five isoforms of β-tubulin, and thus the antibody recognizes all five isoforms of β-tubulin (β1- β5).[2] The antibody localizes β-tubulin in human,[4] bovine,[5] rat,[6] mouse,[3], sea urchin,[7] and plant.[8]

Monoclonal Anti- β-Tubulin-FITC may be used for the detection and localization of β-tubulin using immunocytochemical and immunohistochemical assays.

Tubulin is the major building block of microtubules. This intracellular cylindrical filamentous structure is present in almost all eukaryotic cells. Microtubules function as structural and mobile elements in mitosis, intracellular transport, flagellar movement, and in the cytoskeleton. Tubulin is a heterodimer, which consists of α-tubulin and β-tubulin; both subunits have a molecular weight of 55 kDa and share considerable homology. The most studied tubulins have been isolated from vertebrate brains. The microtubules can be viewed in immunofluorescent microscopy, which enables the observation of the intracellular organization of proteins that are in the form of a supramolecular structure.[9-11]

## Reagent

Supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

F/P Molar Ratio: 3.0 - 5.0

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet 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 at –20 °C in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. 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.

## Product Profile

Direct Immunofluorescence: A working dilution of 1:25 was determined using cultured chicken fibroblasts or BHK cells.

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

## Direct Immunofluorescence Labeling of Cultured Cells

### Materials

- Coverslips
- Cells in DMEM medium + 10% Fetal Calf Serum (Example., chicken fibroblasts, BHK cells)
- 10 mM phosphate buffered saline (PBS), pH 7.4, without preservative, Cat. No. P3813
- PBS with 1% BSA (diluent), Cat. No. P3688
- Absolute methanol, cooled to –20 °C
- Acetone, Analytical grade, cooled to –20 °C
- Aqueous mounting media
- Monoclonal Anti- β-Tubulin-FITC

### Cell Growth and Fixation

1. Collect cells from tissue culture dish at a stage of almost confluency, wash with medium and seed on to coverslips. Seed 1-2 x $10^4$ cells per coverslip and grow cells in incubator for 2-3 days. Do not change medium.
2. Remove coverslips from incubator, aspirate medium.
3. Wash twice with PBS; 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 twice for 10 seconds with cold acetone, aspirate.
6. Wash 2x with PBS. Rehydrate in PBS for at least 30 minutes prior to labeling with antibody.

### Direct Immunofluorescence Labeling

1. Dilute antibody conjugate in PBS containing 1% BSA. Add enough diluted antibody conjugate to cover the cell layer and incubate coverslips for 60 minutes at room temperature.
2. Wash 3x with PBS, 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. Read under UV fluorescence microscope. Mounted preparations can be stored in the dark at 2-8 °C.

### Notes

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

## References


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