V. 3.0

Tubulin Protein (TAMRA Rhodamine Labeled, from porcine brain)
Cat. # TL590M

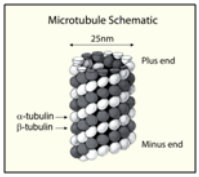
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

Tubulin is composed of a heterodimer of two closely related 55 kDa proteins called alpha and beta tubulin. The two proteins are encoded by separate genes, or small gene families, whose sequences are highly conserved throughout the eukaryotic kingdom. Consequently, tubulin isolated from porcine brain tissue is highly homologous to tubulin isolated from the majority of eukaryotic sources. This fact results in the technical benefit that porcine tubulin can be used to assay proteins originating from many diverse species.

Tubulin polymerizes to form structures called microtubules (MTs). When tubulin polymerizes it initially forms protofilaments, MTs consist of 13 protofilaments and are 25 nm in diameter. Each µm of MT length is composed of 1650 heterodimers (1). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 1).

Figure 1: Microtubule Schematic



Tubulin can polymerize from both ends *in vitro*, however, the rate of polymerization is not equal. It has therefore become the convention to call the rapidly polymerizing end the plus -end and the slowly polymerizing end the minus-end. *In vivo* the plus-end of a microtubule is distal to the microtubule organizing center.

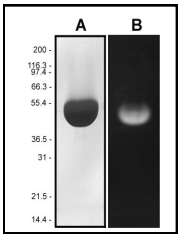
The intrinsic ability of pure tubulin to polymerize *in vitro* is very much a function of the experimental conditions. For example, one can manipulate the polymerization reaction to give microtubules of a particular mean length distribution or create conditions under which tubulin will not polymerize significantly until an enhancer component, such as a polymerization stimulating drug or protein, is added. The propensity of tubulin subunits to assemble into microtubules is dependent upon their affinity for microtubule ends (termed critical concentration [CC]). In order to achieve polymerization the CC needs to be less than the total tubulin concentration. At concentrations above the CC, tubulin will polymerize until the free subunit concentration is equal to the CC value. Because of this parameter, pure tubulin in General Tubulin Buffer will not polymerize significantly at concentrations below 5 mg/ml. If, however, one adds a polymerization enhancer such as 5% glycerol, tubulin polymerization efficiency will approach 90% polymer mass at 37°C after 15-20 minutes. Tubulin polymerization is also a temperature sensitive event, optimal polymerization occurs at 37°C.

Material

Tubulin protein has been purified from porcine brain by an adaptation of the method of Shelanski et al (2). Further purification is achieved by cation exchange chromatography. Tubulin consists of a heterodimer of one alpha and one beta isotype, each isotype is 55 kDa in size. SDS-PAGE analysis shows tubulin running as a 55 kDA species (see Figure 2). Typically, the molar equivalent of tubulin is defined as the heterodimer, which has a molecular weight of 110 kDa.

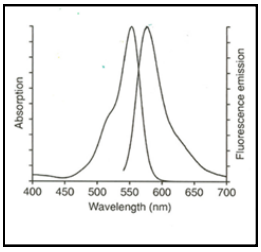
The protein is modified to contain covalently linked rhodamines at random surface lysines. An activated ester of rhodamine [(5-(and 6)-carboxytetramethylrhodamine [TAMRA] succinimidyl ester] is used to label the protein. Labeling stoichiometry is determined by spectroscopic measurement of protein and dye concentrations. Final labeling stoichiometry is 1-2 dyes per protein molecule. The material is guaranteed to contain <10% of free dye and >90% of dye conjugated to tubulin. Rhodamine tubulin has a maximum excitation wavelength of 547 nm and a maximum emission wavelength of 576 nm (see Figure 3) and can be detected using a filter set of 535nm excitation and 585 nm emission. The protein is supplied as a light red lyophilized powder. Each vial contains 20 µg of tubulin. Protein purity was determined to be >99% (see Figure 2).

Figure 2: Purity and Labeling Analysis of Tubulin Protein



Legend: Lane A; 25 µg sample of unlabeled tubulin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Lane B; 20 µg of the same protein sample, after TAMRA, SE conjugation, was run in a 4-20% SDS-PAGE system and photographed directly under UV illumination.

Figure 3: Spectral Scan of Rhodamine Labeled Tubulin



Legend: Labeled tubulin protein was diluted with sterile distilled water and its absorbance and fluorescence spectrum measured. The absorbance peak is at 547 nm and fluorescence at 576nm.

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Storage and Reconstitution

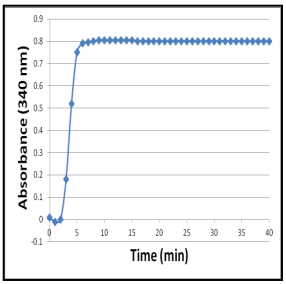
The recommended storage conditions for the lyophilized material is 4°C and <10% humidity in the dark. Lyophilized protein can also be stored desiccated at -70°C, protected from light. In both cases the protein is stable for 1 year. Prior to reconstitution, it is recommended to briefly centrifuge the tube to pellet the pink tubulin containing powder to the bottom of the tube.

Reconstitute to 10 mg/ml with 2 µl of General Tubulin Buffer (80 mM PIPES pH6.9, 2mM $MgCl_2$, 0.5 mM EGTA) supplemented with 1mM GTP. Use immediately or snap freeze in liquid nitrogen and stored at -70°C (stable for 6 months). NOTE: Reconstituted tubulin MUST be snap frozen in liquid nitrogen prior to storage at –70°C, failure to do this will result in significant loss of activity. Avoid repeated freeze / thaw cycles.

Biological Activity Assay

The biological activity of TL590M is assessed by a tubulin polymerization assay. The ability of tubulin to polymerize into microtubules can be followed by observing an increase in optical density of a tubulin solution at OD_{340nm} (see Figure 4). Under the experimental conditions defined below a 5 mg/ml tubulin solution in General Tubulin Buffer buffer plus 5% glycerol and 1 mM GTP should achieve an OD_{340nm} absorbance reading between 0.95 - 1.3 per cm of light pathlength in 30 minutes at 37°C. The assay volume is 180 µl and assumes a spectrophotometer path-length of 0.8 cm, so the expected OD is 0.78 to 1.1.

Figure 4: Polymerization of Rhodamine-Labeled Tubulin



Legend: Labeled tubulin protein at 5 mg/ml was resuspended in ice cold General Tubulin Buffer plus 5% glycerol and 1 mM GTP. Tubulin was pipetted into a pre-warmed 96 well plate and incubated at 37°C for 40 min. The increase in OD340 over the first 10 min. is indicative of microtubule polymerization.

Product Uses

- Measurement of microtubule dynamics *in vivo* (3).
- Formation of fluorescent microtubules for *in vitro* kinesin driven motility assays (4 and see Application Note #1 and Figure 5)

References

1. Amos, L.A. & Klug A. 1974. *J. Cell Sci.* **14**:523-530
2. Shelanski M.L. et al. 1973. *Proc. Natl. Acac. Sci. USA.* **70**: 765-768.
3. Piehl M. and Cassimeris L. 2003. Organization and dynamics of growing microtubule plus ends during early mitosis. *Mol. Biol. Cell* **14**: 916-925.
4. Hyman A.A. et al. 1992. Microtubule motor activity of a yeast centromere-binding complex. *Nature* **359**: 533-536.

Product Citations/Related Products

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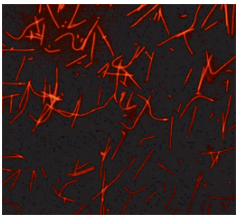
Application Note #1: In vitro Polymerization for Fluorescent Microtubules

Method: (to generate 5-10µm long microtubules in vitro)

1. Briefly centrifuge the labeled tubulin vial to collect the pink powder to the bottom of the tube.
2. Resuspend tubulin in 5µl of General Tubulin Buffer supplemented with 10% glycerol and 1 mM GTP.
3. Incubate at 37°C for 20min. to polymerize the tubulin and form microtubules (MTs).
4. Pipette 0.7µl of a 200µM paclitaxel (taxol) stock into the MT reaction, incubate at 37°C for 5min. This step stabilizes the MTs.
5. Place at room temperature (RT). This is your MT stock that can be stored up to 2 days at RT (not 4°C). Protect MTs from light by wrapping the tube in foil.
6. For microscopic observation: Dilute 1µl of MT stock into 200 µl of 37°C warm General Tubulin Buffer supplemented with 20 µM taxol (20µl of 200µM taxol stock into 180µl of General Tubulin Buffer). The maximal excitation and emission wavelengths of TAMRA and a recommended filter set are given in the Materials section of this datasheet (and see Figure 5).

Figure 5: Microscopic Observation of Rhodamine Labeled MTs



Legend: Rhodamine labeled tubulin was polymerized into microtubules (MTs) as described in Application Note #1. MTs were visualized by fluorescence microscopy using a 100X oil immersion lens and 535 nm excitation, 585 nm emission filters.

Technical Notes: Working with Fluorescent Tubulin

1. Any buffer containing GTP should be kept on ice and used within 1-2h after addition of GTP as GTP will hydrolyse over time. Unused GTP supplemented buffer should be discarded.
2. Tubulin is a labile protein and should be used immediately after resuspension or snap frozen into appropriate aliquots (see Storage and Resuspension section).
3. It is not recommended to snap freeze and store tubulin at concentrations below 10 mg/ml. For applications requiring dilute tubulin, dilutions should be made immediately prior to using the product. Any dilute reagent remaining at the end of the experiment should be discarded as significant loss of activity will result from trying to store the dilute protein at –70°C.