V. 1.4

Tubulin protein
(HiLyte Fluor™ 647 labeled, from porcine brain)
Cat. # TL670M

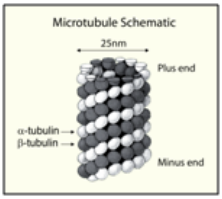
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

About Tubulin

Tubulin is composed of a heterodimer of two closely related 55 kDa proteins called α and β 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 (2). MTs are highly ordered structures that have an intrinsic polarity (Figure 1a).

Figure 1a: Microtubule Schematic



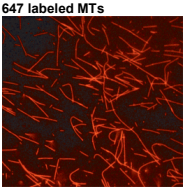
Tubulin can polymerize from both ends *in vitro*; however, the rate of polymerization is not equal. It has therefore become the convention to call the rapidly polymerizing end the plus-end and the slowly polymerizing end the minus-end. *In vivo,* the plus-end of a MT is distal to the MT organizing center. 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 MTs 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 MTs is dependent upon their affinity for MT 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 100% polymer mass at 37° C after 15-20 minutes. Tubulin polymerization is also a temperature sensitive event; optimal polymerization occurs at 37°C.

Stoichiometry of protein Labeling

To pass quality control, the stoichiometry of labeling must be between 0.2-0.7 dye molecules per tubulin heterodimer. The calculated stoichiometry for a specific Lot is described on page 1.

Material

Figure 1b: HiLyte Fluor™ 647 labeled MTs



Porcine brain tubulin has been modified to contain covalently linked HiLyte Fluor™ 647 at random surface lysines. An activated ester of the fluorochrome was used to label the protein. Labeling stoichiometry was determined by spectroscopic measurement of protein and dye concentrations (dye extinction coefficient when protein bound is 240,000$M^{-1}cm^{-1}$). Final labeling stoichiometry is 0.2-0.7 dyes per tubulin heterodimer. HiLyte Fluor™ 647 labeled tubulin can be detected using a filter set of 610-640 nm excitation and 670-700 emission (Fig. 3). Each tube contains 20 µg of protein, supplied as a light blue lyophilized powder. Figure 1b indicates the product after polymerization into MTs. HiLyte Fluor™ 647 is a trademark of Anaspec Inc. (CA, USA).

Storage and Reconstitution

The recommended storage conditions for the lyophilized material is 4°C and <10% humidity in the dark. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C and is stable for 1 year. To reconstitute the protein, briefly centrifuge to collect the product at the bottom of the tube. This should be visible as a light blue pellet. Resuspend to 10 mg/ml with 2 µl of either water or General Tubulin Buffer plus GTP (G-PEM) (see Application Detail for choosing between these options). The concentrated protein in G-PEM can be snap frozen in liquid nitrogen and stored at -70°C (stable for 6 months). **NOTE:** It is very important to snap freeze the tubulin in liquid nitrogen as other methods of freezing will result in significantly reduced activity. Defrost rapidly by placing in a room temperature wate bath for 1min. Avoid repeated freeze / thaw cycles.

Purity

The protein used for TL670M was determined to be >99% pure tubulin by Coomassie stained SDS-PAGE analysis (Figure 2A). To determine whether any free dye is present, a 25 µg sample of protein was run on an SDS gel and photographed under orange light (Figure 2B). Unincorporated dye would be visible in the dye front.

Figure 2 - Purity

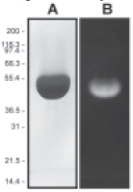


Figure 2. Lane A; 25 µg sample of unlabeled tubulin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue (A). Lane B; 20 µg of the same protein sample after HiLyte Fluor™ 647 conjugation was run in a 4-20% SDS-PAGE system and photographed directly under orange illumination.

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Application details:

Application #1. *In vitro* polymerization for fluorescence microscopy (Figure 1b).
For example: kinesin/dynein, nanotechnology, and MAP binding or MT length measurements.

Reagents
1. 20µg vial of HiLyte Fluor™ 647 labeled porcine tubulin (Cat. # TL670M)
2. 1ml of fresh ice cold G-PEM buffer with 10% (w/v) glycerol (Cat. #s BST01, BST05, and BST06)
3. 1ml of fresh 37°C warm G-PEM buffer with 30% (w/v) glycerol and (Cat. #s BST01, BST05, BST06, and TXD01)
4. 100 µl of 2mM TXD01 in DMSO (RT)

Equipment
1. Fluorescence microscope with Ex:630 / Em: 670 filter set.

Method (to generate 5-10 µm long MTs, for examples see Figure 1b)
1. Briefly centrifuge labeled tubulin vial to collect powder to the bottom of the tube.
2. Place vial and G-PEM plus 10% glycerol buffer on ice.
3. Resuspend tubulin in 5 µl of buffer. Optional: centrifuge 14,000 x g, 4°C, 10 min and pipette supernatant into a fresh tube on ice, this will reduce "aster" type aggregates seen under the microscope.
4. Place vial at 37°C for 20 min.
5. Dilute 2 µl of TXD01 into 18 µl of G-PEM plus 10% glycerol buffer, mix and place at RT (200 µM).
6. Pipette 0.7 µl of 200 µM taxol stock into the microtubule reaction, incubate at 37°C for 5 min and place at RT wrapped in foil. This is your MT stock that can be stored upto 2 days at RT (not 4°C).
7. For microscopic observation: Dilute 1 µl of MT stock into 200 µl of 37°C warm G-PEM buffer plus 30% (w/v) glycerol and 20 µM taxol. (20 µl of 200 µM taxol stock into 180 µl of G-PEM plus 30% glycerol buffer).
8. Immediately pipette 5 µl onto a glass slide and place cover-slip on top. Optional #1 for real time motor assays: add an antifade solution to the mixture prior to visulaizing (e.g. 100mM glucose, 10 units/ml glucose oxidase and 0.5 mM BME or DTT). Optional #2 for completed reactions (e.g. MT crosslinking proteins): Add 0.5% gluteraldyde to G-PEM plus 30% glycerol buffer prior to diluting MTs.

Application #2. *In vivo* cell microinjection studies
For example: Cellular motility, invasion, growth and division or vesicle tracking studies.

Reagents
1. 20 µg vial of labeled tubulin (Cat. # TL670M)
2. 1 ml of sterile ice cold Milli-Q or nanopure water

Equipment
1. Fluorescence microscope with Ex:630 / Em: 670 filter set and 37°C thermo-regulated stage.
2. Microinjection appartus or cellular protein-loading method

Method
1. Briefly centrifuge tubulin vial to collect powder to the bottom of the tube.
2. Place vial and water on ice.
3. Resuspend tubulin in 5µl of ice cold water. Optional: centrifuge 14,000 x g, 4°C, 10 min and pipette supernatant into a fresh tube on ice, this will reduce potential injection needle blocking.
4. Draw solution up into injection needle or prepare protein loading method sample.
5. Inject cell with 0.25 to 0.5 nl of solution, or apply protein loading reagent.
6. Visualize under microscope with low light levels and a 37°C warmed stage.

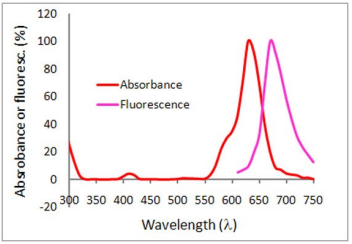

Figure 3. Spectral Scans of HiLyte Fluor™ 647 labeled tubulin protein. Labeled tubulin protein was diluted with sterile distilled water and its absorbance and fluorescence spectrum was scanned between 250 and 750 nm. Absorbance peaks at 640 nm and fluorescence at 670nm.

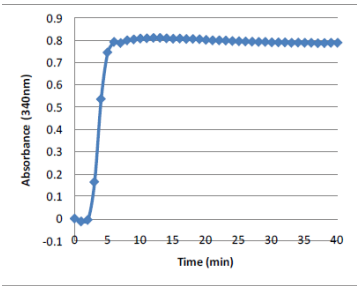

Figure 4. Polymerization of HiLyte Fluor™ 647 labeled tubulin protein. Labeled tubulin protein at 5 mg/ml was resuspended in ice cold G-PEM plus 5% (v/w) glycerol. Tubulin was pipetted into a pre-warmed 96-well plate and incubated at 37°C for 40min. The increase in OD340nm over the first 10 min is indicative of micro-tubule polymerization.

Product Uses
- Measurement of microtubule dynamics *in vivo*.
- Fluorescent microtubules for *in vitro* kinesin driven motility assays.

Product Citations/Related Products
For the latest citations and related products please visit www.cytoskeleton.com.