V. 5.0

Tubulin protein
(HiLyte Fluor™ 488 labeled, from porcine brain)
Cat. # TL488M

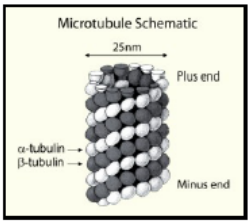
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

Tubulin is composed of a heterodimer of two closely related 55 kDa proteins called 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 (1). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 1).

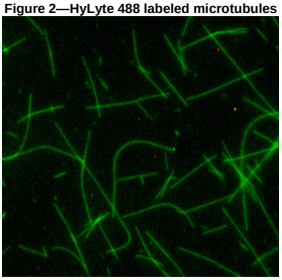
Figure 1. Microtubule Schematic



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 100% polymer mass at 37°C after 15-20 minutes. Tubulin polymerization is also a temperature sensitive event, optimal polymerization occurs at 37° C.

Material

Figure 2—HyLyte 488 labeled microtubules



Porcine brain tubulin has been modified to contain covalently linked HiLyte Fluor™ 488 (2) 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 76,000M-1cm-1). Final labeling stoichiometry is 0.8-1.5 dyes per tubulin heterodimer. HiLyte Fluor™ 488 labeled tubulin can be detected using a filter set of 502 nm excitation and 527 emission. Each tube contains 20 µg of protein, supplied as an orange lyophilized powder. Figure 2 indicates the product after polymerization into microtubules.
HiLyte Fluor™ 488 is a trademark of Anaspec Inc. (CA, USA). (2)

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 orange colored pellet, resuspend to 10 mg/ml with 2 µl of General Tubulin Buffer plus GTP (80 mM PIPES, 0.5 mM EGTA, 2 mM $MgCl_2$, 1 mM GTP pH 6.9). The concentrated protein 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 water bath for 1min. Avoid repeated freeze / thaw cycles.

Purity

The protein used for TL488M was determined to be >99% pure tubulin by coomassie stained SDS-PAGE analysis (Figure 3A). To determine whether any free dye is present, a 25 µg sample of protein was run on an SDS gel and photographed under UV light (Figure 3B). Unincorporated dye would be visible in the dye front. No fluorescence was detected in the dye front indicating that all free dye has been removed from the labeling reaction.

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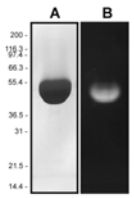
Figure 3. Lane A; 25 µg sample of unlabeled tubulin protein was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue (A). Lane B; 20 µg of the same protein sample after HiLyte 488 conjugation was run in a 4-20% SDS-PAGE system and photographed directly under UV illumination.

Figure 3— Purity



Application details:

Application #1. In vitro polymerization for fluorescence microscopy (Figure 2).
For example: kinesin/dynein, nanotechnology and MAP binding or MT length measurements.

Reagents
1. 20µg vial of HiLyte Fluor™ 488 labeled porcine tubulin (Cat. # TL488M)
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:480 / Em: 550 filter set.

Method (to generate 5-10µm long microtubules, for examples see Figure 2)
1. Briefly centrifuge HiLyte Fluor 488 labeled tubulin vial to collect powder to the bottom of the tube.
2. Place HiLyte Fluor 488 labeled tubulin vial and G-PEM plus 10% glycerol buffer on ice.
3. Resuspend HiLyte Fluor 488 labeled tubulin in 5µl of buffer. Optional: centrifuge 14,000xg, 4°C, 10min 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 20min.
5. Dilute 2µl of TXD01 into 18µl of G-PEM plus 10% glycerol buffer, mix and place at RT (200µM).
6. Pipette 0.7µl of 200µM taxol stock into the microtubule reaction, incubate at 37°C for 5min and place at RT wrapped in foil. This is your MT stock that can be stored upto 2 days at RT.
7. For microscopic observation: Dilute 1µl of MT stock into 200 µl of 37°C warm G-PEM buffer plus 30% (w/v) glycerol and 20 µM taxol. (20µl of 200µM taxol stock into 180µl of G-PEM plus 30% glycerol buffer).
8. Immediately pipette 5µl onto a glass slide and place coverslip on top. Optional #1 for real time motor assays: add an antifade solution to the mixture prior to visualizing (e.g. 100mM glucose, 10units/ml glucose oxidase and 0.5mM 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. 20µg vial of HiLyte Fluor™ 488 labeled labeled porcine tubulin (Cat. # TL488M)
2. 1ml of sterile ice cold Milli-Q or nanopure water

Equipment
1. Fluorescence microscope with Ex:480 / Em: 550 filter set and 37°C thermo-regulated stage.
2. Microinjection appartus or cellular protein-loading method

Method
1. Briefly centrifuge HiLyte Fluor 488 labeled tubulin vial to collect powder to the bottom of the tube.
2. Place HiLyte Fluor 488 labeled tubulin vial and water on ice.
3. Resuspend tubulin in 5µl of ice cold water. Optional: centrifuge 14,000xg, 4°C, 10min 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.

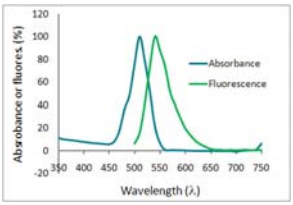


Figure 4. Absorbance and fluorescence scan of HiLyte Fluor™ 488 labeled tubulin protein. Labeled tubulin protein was diluted with sterile distilled water and its absorbance and excitation spectra were scanned between 250 and 750 nm and 500 and 750 resp. Absorbance peaks at 488 nm and fluorescence at 550 nm.

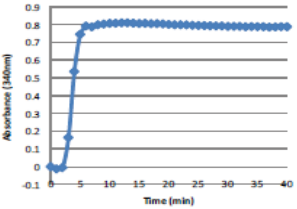


Figure 5. Polymerization of HiLyte Fluor™488 labeled tubulin protein. Labeled tubullin protein at 5mg/ml was resuspended in ice cold G-PEM plus 5% (v/w) glycerol. Tubulin was pipetted into a pre-warmed 96-well plate and incubated at 37°C for 40min. The increase in OD340nm over the first 10 min is indicative of microtubule polymerization.

Product Uses
- Measurement of microtubule dynamics in vivo.
- Fluorescent microtubules for in vitro kinesin driven motility assays (see also Cat. # BK027).

References
1. Amos, LA. & Klug A. 1974. J.Cell Sci. 14: 523-530.

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