About Tubulin

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 (2). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 3).

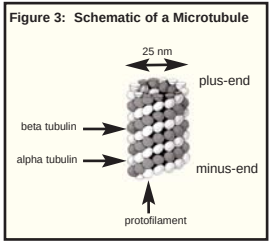

Figure 3: Schematic of a Microtubule

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.

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Tubulin (>99% pure)

Isolated from porcine brain

Cat. # T240 1 x 1 mg
Lot # 071

Store at 4°C (desiccated) or at -70°C

Material

Tubulin protein has been purified from porcine brain by an adaptation of the method of Shelanski et al. (1), Further purification to >99% purity was achieved by cation exchange chromatography. Tubulin is supplied as a white lyophilized powder. Tubulin consists of a heterodimer of one alpha and one beta isotype, each tubulin isotype is 55 kDa in size, SDS-PAGE analysis shows tubulin running as a 55 kDa species (see Figure 1). Typically, the molar equivalent of tubulin is defined as the heterodimer which has a molecular weight of 110 kDa.

Storage and Reconstitution

The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C.

The protein should be reconstituted to 10 mg/ml with General Tubulin Buffer supplemented with 1 mM GTP. Snap freeze "experiment sized" aliquots in liquid nitrogen and store at -70°C. Reconstituted T240 is stable for 6 months at -70°C. **Reconstituted T240 MUST be snap frozen in liquid nitrogen prior to storage at -70°C, failure to do this will result in significant loss of activity**.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. T240 is determined to be >99% pure tubulin (mol. wt 55 kDa). Note: due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker band.

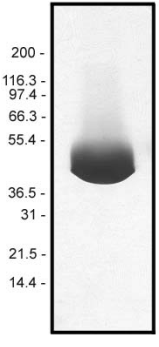

Figure 1. T240 Protein Purity Determination A 50 µg sample of T240 protein was separated by electrophoresis in a 4-20% SDS-PAGE system, and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Molecular weight markers are from Invitrogen (Mark 12). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker band.

Biological Activity Assay

The biological activity of T240 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 OD340 nm (see Figure 2). 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 OD340 nm absorption 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 pathlength of 0.8 cm, so the expected OD is 0.78 to 1.1. NOTE: when using a microtiter plate compatible spectrophotometer the readings are taken from the top of the plate and therefore the volume of your reaction will directly influence the pathlength. Cytoskeleton Inc. highly recommends the use of a 1/2 area well plate (Corning Cat. # 3696) for optimal polymerization signal in this assay.

Reagents

1) Tubulin protein (Cat. # T240)
2) GTP stock (100 mM) (Cat. # BST06)
3) General Tubulin Buffer (Cat. # BST01); 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA
4) Tubulin Glycerol Buffer (Cat. # BST05); 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 60% glycerol.

Equipment

1) Temperature regulated spectrophotometer set on kinetic mode at 340 nm.
2) Half area well plate (Corning Cat. # 3696).

Method

1) Aliquot 907 µl of ice cold General Tubulin Buffer into an microfuge tube and add 83 µl of Tubulin Glycerol Buffer and 10 µl of 100 mM GTP stock to give a final buffer composition of General Tubulin Buffer containing 5% glycerol and 1 mM GTP. Keep this on ice and use within 2-4 hours.
2) Resuspend a 1 mg vial of T240 with 200 µl of the ice cold 5% glycerol buffer.
3) Leave the protein on ice for 2-3 minutes to dissolve the tubulin protein pellet.
4) The vial of protein should then be mixed well with a pipette to make sure that the protein has resuspended evenly.
5) Tubulin is a labile protein and should be used immediately after resuspension. Keep tubulin on ice prior to beginning the polymerization reaction.
6) For a standard 96 well plate assay you should transfer 180 µl of the resuspended T240 (at 4°C) into a microtiter plate that has been pre-warmed to 37°C. Cytoskeleton Inc. highly recommends the use of a 1/2 area well plate (Corning Cat. # 3696) for optimal polymerization in this assay.
7) Measure tubulin polymerization by taking readings every 30 seconds at 340 nm and 37°C for 45 minutes to 1 hour total. You do not need to designate a blank well, all wells can be individually blanked at the beginning of the assay or data can be transferred to Excel.
7) Note: Temperature is an extremely important parameter for tubulin polymerization, temperatures cooler than 37°C will significantly decrease the rate and final OD reading of a polymerization reaction. If tubulin is aliquoted into a cool plate (or room temperature plate) there will be a much longer nucleation phase (Phase I, Figure 2).
8) Figure 2 shows the results of polymerizing T240 under the conditions described above. It should be noted that you may wish to optimize your particular assay by either altering the protein concentration and/or the final reaction volume. For example, if you wish to examine polymerization enhancers such as taxol, it is recommended to reduce the tubulin concentration to 1 to 3 mg/ml and polymerize in G-PEM buffer minus glycerol. These conditions will result in a very slow and shallow polymerization curve for the "no compound" control. In this case, efficient polymerization is achieved by addition of an enhancer such as taxol (5 - 10 µM final concentration).

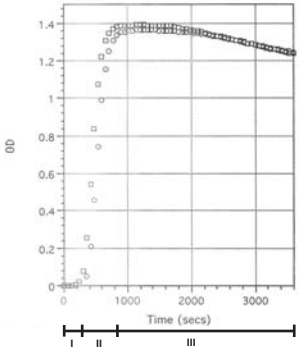

Figure 2: Tubulin Polymerization Assay

Polymerizations were carried out as indicated in the Method section. Briefly, the polymerization reaction contains 180 µl of 5 mg/ml tubulin in 80 mM PIPES pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$, 5% glycerol, 1 mM GTP. Polymerization was started by incubation at 37°C and followed by absorption readings at 340 nm. Under these conditions polymerization reached a maximal OD340 between 0.78 - 1.1 within 30 minutes. In this experimental set up (180 µl volume in a spectrophotometer with a pathlength of 0.8 cm) an OD340 is approximately equal to 1 mg per ml of polymer mass. Thus, under the conditions described, approximately 90% of the tubulin is polymerized. The three phases of polymerization are shown, I (nucleation), II (growth), III (steady state). The average Vmax of the polymerization is 150 milli OD units per minute (mOD/min). Duplicate reactions were performed.

Product Uses

Recommended for IC50 & EC50 determinations for anti-tubulin ligands.

Recommended for examining tubulin / protein interactions.

References

1) Shelanski ML, et al. 1973. Proc. Natl. Acad. Science USA. 70: 765-768
2) Amos, LA. & Klug A. 1974. J. Cell Sci. 14: 523-530.

Related Products

Cytoskeleton Inc. is the leading supplier of purified tubulins, visit our web site or call for information on tubulin products currently available. A selected list of tubulin related products is given below;

* Tubulin polymerization assay (absorbance, >97% tubulin)	Cat. # BK004P
* Tubulin polymerization assay (absorbance, >99% tubulin)	Cat. # BK006P
* Tubulin polymerization assay (fluorescence based)	Cat. # BK011P
* Microtubule binding protein assay	Cat. # BK029
* HTS tubulin (>97% pure, recommended for primary screens)	Cat. # HTS03
* Biotin tubulin	Cat. # T333P
* Cancer cell tubulin (source; HeLa cells)	Cat. # H001
* Cancer cell tubulin (source; MCF-7)	Cat. # H005
* Biotin cancer cell tubulin	Cat. # H003
* Rhodamine tubulin	Cat. # TL590M
* Pre-formed microtubules	Cat. # MT002
* MAP-rich tubulin	Cat. # ML116
* General Tubulin Buffer	Cat. # BST01
* Tubulin Glycerol Buffer	Cat. # BST05
* GTP stock (100 mM)	Cat. # BST06
* Paclitaxel stock (2 mM)	Cat. # TXD01