

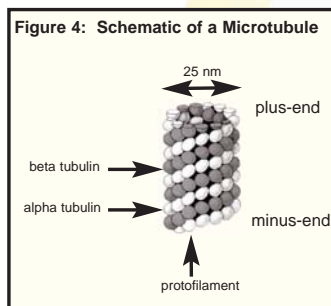
## Related Products

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. # BK004
* Tubulin polymerization assay (absorbance, >99% tubulin)	Cat. # BK006
* Tubulin polymerization assay (fluorescence based)	Cat. # BK011
* Microtubule binding protein assay	Cat. # BK029
* Tubulin protein (>99% pure, Bovine brain, lyophilized)	Cat. # TL238
* Tubulin protein minus glycerol (>99% pure, Bovine brain)	Cat. # T238
* Tubulin protein (>99% pure, Porcine brain)	Cat. # T240
* HTS tubulin (>97% pure, recommended for primary screens)	Cat. # HTS02
* Biotin tubulin	Cat. # T333
* Cancer cell tubulin (source; HeLa or MCF-7cells)	Cat. # H001 or H005 resp.
* Rhodamine tubulin	Cat. # TL331M
* MAP-rich tubulin	Cat. # ML113
* Pre-formed microtubules	Cat. # MT001

## 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 bovine brain tissue is highly homologous to tubulin isolated from the majority of eukaryotic sources. This fact results in the technical benefit that bovine 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  $\mu\text{m}$  of MT length is composed of 1650 heterodimers (3). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 4).



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.



## Tubulin > 99% pure (plus glycerol)

**Bovine brain**

**Cat. # T237**

**Lot # 287 Amount per tube: 250  $\mu\text{g}$**

**Store at -70°C**

### Material

Tubulin protein has been purified from bovine brain by an adaptation of the method of Shelanski et al. (1). Further purification to >99% purity was achieved by cation exchange chromatography. Bovine brain tubulin is supplied at 10 mg/ml in 80 mM PIPES pH 6.9, 2.0 mM  $\text{MgCl}_2$ , 0.5 mM EGTA, 1 mM GTP (G-PEM) and 10% glycerol. 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

It is recommended that T237 be stored at -70°C, where it is stable for 6 months.

The protein should be rapidly thawed in a room temperature water bath, immediately transferred to ice and aliquoted into "experiment sized" amounts. The aliquots MUST be snap frozen in liquid nitrogen and stored at -70°C. **T237 MUST be snap frozen in liquid nitrogen prior to storage at -70°C, failure to do this results in significant loss of activity. Tubulin will be active for only 1 week if stored at -40°C.**

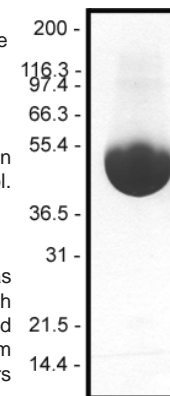
### Unit Definition

One unit of tubulin is defined as 5.0 mg of purified protein (as determined by the method of Bradford (2)).

### Purity

Purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. T237 is determined to be >99% pure tubulin (mol. wt 55 kDa).

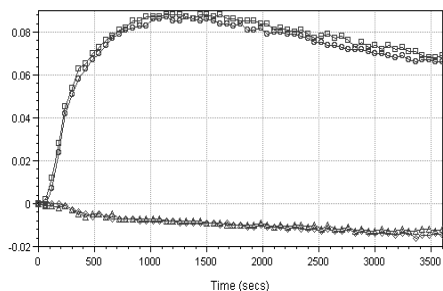
**Figure 1. T237 Protein Purity Determination** A 50  $\mu\text{g}$  sample of T237 was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat. # ADV02). Molecular weight markers are from Invitrogen (Mark12). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker band.



To Place Orders Tel: (303)-322-2254, Fax: (303)-322-2257, E-mail: [cserve@cytoskeleton.com](mailto:cserve@cytoskeleton.com)  
For Technical Assistance E-mail: [tservice@cytoskeleton.com](mailto:tservice@cytoskeleton.com). Cytoskeleton Inc. Denver, CO. USA.

**WWW.CYTOSKELETON.COM**

page 4



**Figure 2. Tubulin polymerization in the presence or absence of taxol.** A 10 mg/ml stock of pure tubulin (in G-PEM buffer plus 10% glycerol) was diluted in G-PEM buffer to a final concentration of 1.1 mg/ml (10  $\mu$ M tubulin). 100  $\mu$ l samples were incubated in wells of a 1/2 area 96-well plate at 37°C with or without 10  $\mu$ M taxol. Tubulin polymerization was observed by measuring the absorbance at 340nm over time. With a pathlength of 1 cm, an increase in optical density of 0.2 is approximately equal to a polymer mass of 1 mg/ml. A 100  $\mu$ l sample volume is equivalent to a 0.5 cm pathlength. Hence, in the presence of 10  $\mu$ M taxol all of the tubulin is polymerized, while in its absence the critical concentration of the protein is not sufficient to allow polymerization. Duplicate reactions were performed.

### Biological Activity Assay

The biological activity of T237 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 3). 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 - 1.30 per cm pathlength in 30 min at 37°C. In Figure 3, the assay volume is 180  $\mu$ l with a spectrophotometer pathlength of 0.8 cm in a 1/2 area 96 well plate, resulting in OD340 nm change of 0.75-1.10. **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 96 well plate (Corning Cat. # 3696) for optimal polymerization signal in this assay.

### Reagents

- 1) Tubulin protein (Cat. # T237)
- 2) GTP stock (100 mM) (Cat. # BST06)
- 3) General Tubulin Buffer (Cat. # BST01); 80 mM PIPES pH 6.9, 2 mM MgCl<sub>2</sub>, 0.5 mM EGTA

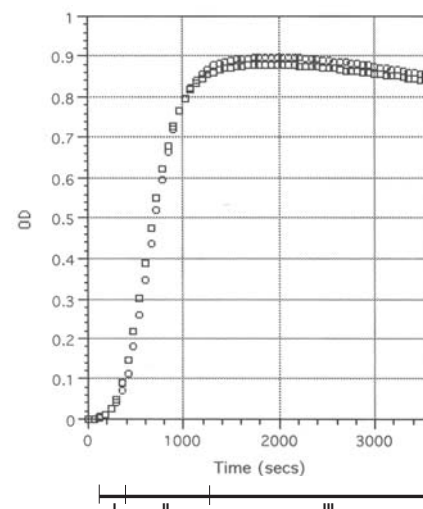
### Equipment

- 1) Temperature regulated spectrophotometer set on kinetic mode at 340 nm.
- 2) 1/2 area well plate (Corning Cat. # 3696 or 3697).

### Method

- 1) Aliquot 990  $\mu$ l of ice cold General Tubulin Buffer into an microfuge tube and add 10  $\mu$ l of 100 mM GTP stock to give a final buffer composition of General Tubulin Buffer containing 1 mM GTP (G-PEM buffer). Keep this on ice and use within 2-4 h.
- 2) Quickly thaw 4 x 250  $\mu$ g tubes of T237 in a room temperature waterbath and immediately transfer to ice. Dilute to 5 mg/ml with ice cold G-PEM buffer. The tubulin is now in G-PEM plus 5% (v/v) glycerol. Centrifuge at 14,000 x g for 10 min at 4°C.
- 3) For a standard 96 well plate assay, transfer 180  $\mu$ l of the tubulin supernatant into a microtiter plate that has been pre-warmed to 37°C. It is essential to use a 1/2 area well plate for optimal polymerization in this assay
- 4) Measure tubulin polymerization by taking readings every 30 s at 340 nm and 37°C for 45 min to 1 h 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™.

- 5) 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 at the rate of 5% loss in polymer per degree Celsius. If tubulin is aliquoted into a cool plate (or room temperature plate) there will be a much longer nucleation phase (Phase I, Fig. 3).
- 6) Figure 3 shows the results of polymerizing T237 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 1-3 mg/ml. In this case, efficient polymerization is achieved by addition of an enhancer such as taxol (5-10  $\mu$ M final concentration), see Figure 2.



**Figure 3: Tubulin Polymerization Assay**

Polymerizations were carried out as indicated in the Method section. Briefly, the polymerization reaction contains 180  $\mu$ l of 5 mg/ml tubulin in 80 mM PIPES pH 6.9, 0.5 mM EGTA, 2 mM MgCl<sub>2</sub>, 5% (v/v) glycerol, 1 mM GTP. Polymerization was started by incubation at 37°C and followed by absorbance readings at 340 nm. Under these conditions polymerization reached a maximal OD340 between 0.75-1.1 within 30 min. In this experimental set up (180  $\mu$ l volume in a spectrophotometer with a pathlength of 0.8 cm) an OD340 of 0.16 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). 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) Bradford, M., 1976. *Analytical Biochemistry*, 72, 248.
- 3) Amos, LA. & Klug A. 1974. *J. Cell Sci.* 14: 523-530.

### Select T237 Citations

- 1) Farah, C. A. et al. 2005. *J Biol Chem*. 280: 9439-9449.
- 2) Hayashi, I and Ikura, M. 2003. *J Biol Chem*. 278:36430-4.
- 3) Segerman, B., et al. 2003. *J Cell Sci*. 116: 197-205.