

Tubulin protein (>99% pure)

Source: Porcine Brain

Cat. # T238P

Lot # 041

Upon arrival store at -70°C

See data sheet for detailed storage recommendations

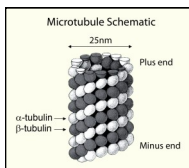
Background Information

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.

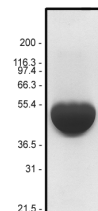


The intrinsic ability of pure tubulin to polymerize *in vitro* is very much a function of the experimental conditions. For example, one can manipulate the polymerization reaction to give microtubules of a particular mean length distribution or create conditions under which tubulin will not polymerize 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. (1). Further purification to >99% purity was achieved by cation exchange chromatography. Porcine brain tubulin is supplied at 10 mg/ml in 80 mM PIPES pH 6.9, 2.0 mM MgCl_2 , 1 mM EGTA and 1 mM GTP (G-PEM). 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 Fig. 2). Typically, the molar equivalent of tubulin is defined as the heterodimer which has a molecular weight of 110 kDa.

Figure 2. Purity Analysis of Tubulin Protein.



Legend: A 50 μg sample of T238P 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.

Storage

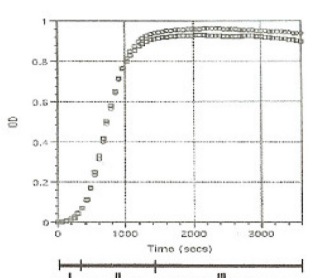
It is recommended that T238P be stored at -70°C , where it is stable for 6 months. The protein should be rapidly thawed in a room temperature water bath, immediately transferred to ice and aliquoted into "experiment sized" amounts. Snap freeze aliquots in liquid nitrogen and store at -70°C . **Aliquots of T238P 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 .**

Biological Activity Assay

The biological activity of T238P 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 $\text{OD}_{340\text{nm}}$ (see Figure 3). Under the experimental conditions defined below a 5 mg/ml tubulin solution in General Tubulin Buffer plus 5% glycerol and 1 mM GTP should achieve an $\text{OD}_{340\text{nm}}$ 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 0.75 to 1.0. NOTE: when using a microtiter plate compatible spectrophotometer the readings are taken from the top of the plate and therefore the volume of the reaction will directly influence the path-length. Cytoskeleton Inc. highly recommends the use of a 1/2 area well plate (Corning Cat. # 3696) for optimal polymerization signal in this assay.

Figure 3. Tubulin Polymerization Assay Results

Reagents



Polymerizations were performed as indicated in the method. The three phases of polymerization are shown. I (nucleation), II (growth), III (steady state). An OD 360 of approximately 1.0 indicates >90% polymerization.

- 1) Tubulin protein (Cat. # T238P)
- 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) 1/2 area 96-well plate (Corning Cat. # 3696 or 3697).

Method

- 1) Add 824 μ l of ice cold General Tubulin Buffer into a centrifuge tube with 166 μ l of Tubulin Glycerol Buffer and 10 ml of 100 mM GTP stock to give a final buffer composition of General Tubulin Buffer containing 10% (v/v) glycerol and 1 mM GTP (G-PEM + 10% glycerol). Keep on ice and use within 2-4 h.
- 2) Quickly thaw required number of tubes of T238P in a room temperature waterbath and immediately transfer to ice. Pool and dilute to 5 mg/ml with an equal volume of G-PEM plus 10% (v/v) glycerol and centrifuge at 14,000 x g for 10 min at 4°C.
- 3) For a standard 96 well plate assay, transfer 180 μ l of the Tubulin into a microtiter plate that has been pre-warmed to 37°C. It is essential to use a 1/2 area 96-well plate for optimal signal generation 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 an Excel™ template (contact tservice@cytoskeleton.com for a copy).
- 5) Figure 3 shows the results of polymerizing T238P under the conditions described above.

Important Technical Notes when Working with Tubulin protein

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). Freeze/thaw cycles should be avoided. Keep tubulin on ice prior to beginning the polymerization reaction.
3. 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 3).
4. Polymerization conditions can be altered to optimize a given assay requirement. For example, to examine polymerization enhancers such as taxol, it is recommended to reduce the tubulin concentration to 1 to 3 mg/ml and polymerize in General Tubulin Buffer plus 1mM GTP 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 μ M final concentration).

Product Uses

- IC50 and EC50 determinations for anti-tubulin ligands.
- Characterization of tubulin binding proteins.

References

1. Shelanski ML, et al. 1973. *Proc. Natl. Acad. Science USA*. **70**: 765-768
2. Bradford, M. 1976. *Analytical Biochem.* **72**: 248
3. Amos, LA. & Klug A. 1974. *J. Cell Sci.* **14**: 523-530.

Product Citations/Related Products

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