V. 1.1

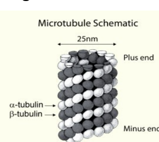
Tubulin > 99% pure (minus glycerol)
Source: Porcine Brain
Cat. # T238P

Upon arrival store at –70°C
See datasheet for detailed storage recommendations

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 (3). MTs are highly ordered structures that have an intrinsic polarity (Fig. 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 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 min. 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 $MgCl_2$, 0.5 mM EGTA and 1 mM GTP (G-PEM). Tubulin consists of a heterodimer of one alpha and one beta isotype and each tubulin isotype is 55 kDa in size. SDS-PAGE analysis shows tubulin running as a 55 kDa species (Fig. 2). Typically, the molar equivalent of tubulin is defined as the heterodimer which has a molecular weight of 110 kDa.

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.**

Unit Definition

One unit of tubulin is defined as 5.0 mg of purified protein (as determined by the method of Bradford (2)). It should be noted that tubulin minus glycerol WILL NOT polymerize efficiently in G-PEM buffer unless high tubulin concentrations are used (10 mg/ml; Fig. 3). Efficient polymerization at low tubulin-glycerol concentrations can be achieved by addition of a polymerization stimulating compound such as glycerol (Fig. 3), taxol (Fig. 4), or DMSO.

Purity

Purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. T238P is determined to be >99% pure tubulin (mol. wt 55 kDa).

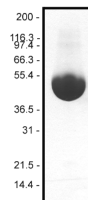


Figure 2. T238P Protein Purity Determination. A 50 µg sample of T238P was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Molecular weight markers are from Invitrogen (Mark12). Note: Due to overloading of the gel, the tubulin band appears to run lower than the 55 kDa marker band.

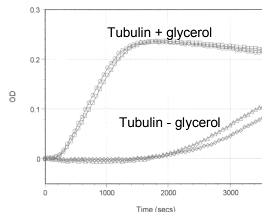


Figure 3. Tubulin polymerization in the presence and absence of glycerol. Samples of 3.3 mg/ml stock of pure tubulin in G-PEM buffer (plus or minus 10% glycerol) were incubated in wells of a 1/2 area 96-well plate at 37°C. Tubulin polymerization was observed

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by measuring the absorbance at 340 nm over time. An increase in optical density of 1.0 is approximately equal to a polymer mass of 5.0 mg/ml with a pathlength of 1 cm. A 100 µl sample volume is equivalent to a 0.5 cm pathlength. Duplicate reactions were performed.

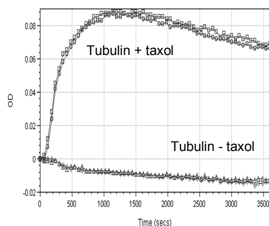

Figure 4. Tubulin polymerization in the presence or absence of taxol. A 10 mg/ml stock of pure tubulin (in G-PEM buffer) was diluted in G-PEM buffer to a final concentration of 1.1 mg/ml (10 µM) tubulin. 100 µl samples were incubated in wells of a 1/2 area 96-well plate at 37°C with or without 10 µM taxol. Tubulin polymerization was observed by measuring the absorbance at 340 nm 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 µl sample volume is equivalent to a 0.5 cm pathlength. Hence, in the presence of 10 µM 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 T238P is assessed by a tubulin polymerization assay. The ability of tubulin to polymerize into MTs can be followed by observing an increase in optical density of a tubulin solution at OD340 nm (see Fig. 5). 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 a 1/2 area 96-well plate (Corning Cat. # 3696). The assay volume is 180 µl 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 (see Fig. 5). 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. # 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 µl of ice cold General Tubulin Buffer into a centrifuge tube with 166 µl of Tubulin Glycerol Buffer and 10 µl 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 250 µg tubes of T238P in a room temperature waterbath and immediately transfer to ice. Pool and dilute to 5 mg/ml with 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 µl 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 as 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. 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. 5).
6. Figure 5 shows the results of polymerizing T238P 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 and polymerize in G-PEM buffer minus glycerol. These conditions will result in a very slow and shallow polymerization curve indicative of slow polymerization (see Fig. 4). In this case, efficient polymerization is achieved by addition of an enhancer such as taxol (5-10 µM final concentration).

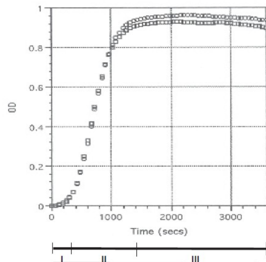

Figure 5: Tubulin Polymerization Assay. Polymerizations were carried out as indicated in the Method section. Briefly, the polymerization reaction contains 180 µl of 5 mg/ml tubulin in 80 mM PIPES pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$, 5% (v/v) glycerol, 1 mM GTP. Polymerization was started by incubation at 37° C and followed by absorbance readings at 340 nm. Under these conditions polymerization reached a maximal OD340 nm between 0.8-1.2 within 30 min, which is equivalent to 90% polymerization. The three phases of polymerization are shown, I (nucleation), II (growth), III (steady state). Duplicate reactions were performed.

Product Uses

- Recommended for IC_{50} & EC_{50} determinations for anti-tubulin ligands.
- Recommended for examining tubulin / protein interactions.

References

1. Shelanski M.L. et al. 1973. *Proc. Natl. Acad. Sci. U.S.A.* 70: 765-768.
2. Bradford M., 1976. *Anal. Biochem.* 72: 248-254.
3. Amos L.A. & Klug A. 1974. *J. Cell Sci.* 14: 523-530.