V. 1.0

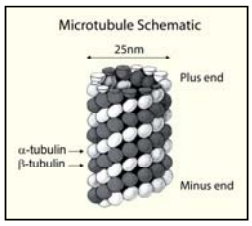
Tubulin protein plus glycerol (>99% pure)
Source: Sheep Brain
Cat. # T234S
Lot #011
Upon arrival store at –70°C

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.

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



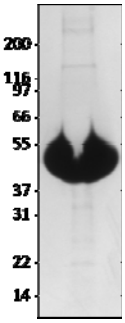
Tubulin can polymerize from both ends *in vitro*, however, the rate of polymerization is not equal. It has therefore become the convention to call the rapidly polymerizing end the plus -end and the slowly polymerizing end the minus-end. *In vivo* the plus-end of a microtubule is distal to the microtubule 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 (80 mM PIPES, pH 7.0, 2 mM MgCl2, 0.5 mM EGTA and 1 mM GTP) 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 sheep brain by an adaptation of the method of Shelanski et al. (2). Further purification to >99% purity was achieved by cation exchange chromatography. Sheep brain tubulin is supplied in 0.55 ml aliquots at 12 mg/ml in 50 mM potassium MES pH 6.8, 0.25 mM $MgCl_2$, 0.5 mM EGTA, 33% glycerol and 0.1 mM GTP. 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 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.



A 100 µg sample of T234S 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. Purity of T234S was determined to be >99%.

Storage

It is recommended that T234S 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 T234S MUST be snap frozen in liquid nitrogen prior to storage at -70°C, failure to do this results in significant loss of activity**.

Biological Activity Assay

The biological activity of T234S 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 OD_{340nm} (see Figure 3). Under the experimental conditions defined below a 6 mg/ml tubulin solution achieve an OD_{340nm} absorbance reading between 1.14 - 1.56 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_{340nm} is 0.91 to 1.25. 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.

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Reagents

1. Tubulin protein (Cat. # T234S)
2. GTP stock (100 mM) (Cat. # BST06)
3. Custom 2x Tubulin Buffer; 80 mM PIPES pH 7.0, 8 mM $MgCl_2$, 1 mM EGTA and 2 mM GTP

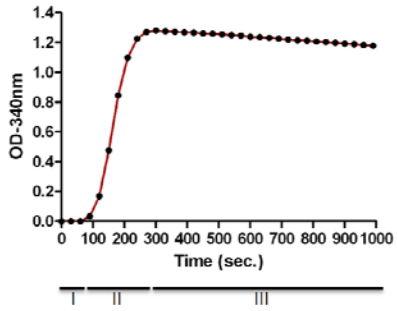
Reagents

1. Temperature regulated spectrophotometer set to 37°C on kinetic mode at 340 nm.
2. 1/2 area 96-well plate (Corning Cat # 3696 or 3697)

Method

1. Quickly thaw one tube of T234S in a room temperature water-bath and immediately transfer to ice.
2. Transfer 300 µl of this stock to a 1.5 ml microcentrifuge tube on ice.
3. Add 300 µl of Custom 2x Tubulin Buffer to the T234S in the microcentrifuge tube and mix well and keep on ice.
4. 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
5. Measure tubulin polymerization by taking readings every 30 seconds at 340 nm and 37°C for 30 min to 1 h total.
6. Figure 3 shows the results of polymerizing T234S under the conditions described above.

Figure 3. Tubulin Polymerization Assay



Polymerizations were carried out as indicated in the Method section. Polymerization was started by incubation at 37°C and followed by absorbance readings at 340 nm. Under these conditions polymerization reached a maximal OD_{340nm} between 1.1-1.25 within 5 min, which is equivalent to >90% polymerization. The three phases of polymerization are shown, I (nucleation), II (growth), III (steady state).

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 thawed or snap frozen into appropriate aliquots (see Storage section). 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_{340nm} 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 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).

Product Uses

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

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References

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

Product Citations/Related Products

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