V. 4.0

Tubulin protein (>99% pure)
Source: Porcine Brain
Cat. # T240

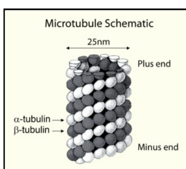
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

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



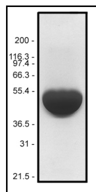
Tubulin can polymerize from both ends *in vitro*, however, the rate of polymerization is not equal. It has therefore become the convention to call the rapidly polymerizing end the plus -end and the slowly polymerizing end the minus-end. *In vivo* the plus-end of a microtubule is distal to the microtubule 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. (2). Further purification was achieved by cation exchange chromatography. 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. The protein is supplied as a white solid. Protein purity was determined to be >99% (see Figure 2).

Figure 2. Purity Analysis of Tubulin Protein.



Legend: 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.

Storage and Reconstitution

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

Reconstitute to 10 mg/ml with General Tubulin Buffer (80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA.) 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.

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 OD_{340nm} (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 OD_{340nm} 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 0.78 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 the reaction will directly influence the path-length. Cytoskeleton Inc. highly

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recommends the use of a 1/2 area well plate (Corning Cat. # 3696) for optimal polymerization signal in this assay.

Polymerization Assay Method

1. Make a 5mg/ml solution of tubulin in 80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 5% glycerol, 1mM GTP as follows;

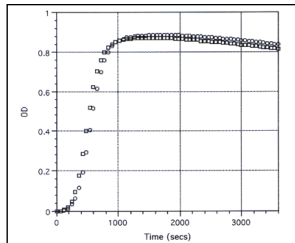
a) Resuspend a lyophilized vial of T240 with 200 µl of the ice cold 5% glycerol buffer (80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 5% glycerol, 1mM GTP) per mg of tubulin protein. For example, a 1mg vial of lyophilized tubulin would be resuspended in 200 µl of buffer. Place on ice.

Or

b) Dilute a 10mg/ml tubulin stock (see Storage and Reconstitution section) to 5 mg/ml with an equal volume of 10% glycerol buffer (80 mM PIPES pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 10% glycerol, 1 mM GTP). Place on ice.

2. Transfer 180 µl of the tubulin solution (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.
3. 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. Typical polymerization assay results for the conditions described above are shown in Figure 3.

Figure 3. Tubulin Polymerization Assay



Legend: Polymerizations were carried out as indicated in the Method. Under these conditions an OD340 of 0.18 is approximately equal to 1 mg per ml of polymer mass. Thus, under the conditions described, > 90% of the tubulin is polymerized. The three phases of polymerization are Phase I: nucleation (0-200 seconds), Phase II: growth (200-1000 seconds) and Phase III: steady state (1000-3600 seconds). Duplicate reactions were performed.

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 and Resuspension 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 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 µM final concentration).

Product Uses

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

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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