V. 1.0

Tubulin MAP-rich
(porcine brain)
Cat. # ML116

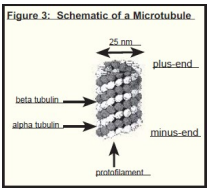
Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

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 µm of MT length is composed of 1650 heterodimers (2). Microtubules are highly ordered structures that have an intrinsic polarity (see Figure 3).

Figure 3: Schematic of a Microtubule



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 min. Tubulin polymerization is also a temperature
sensitive event, optimal polymerization occurs at 37°C.

Material

Tubulin and microtubule associated proteins (MAPs) has been purified from porcine brain by anadaptation of the method of Shelanski et al. (1). Tubulin is supplied as a white lyophilized powder.

The protein composition is approximately 70% tubulin (55 kDa heterodimer) and 30% MAPs (see Figure 1). The MAPs in this product act to stabilize microtubules and to enhance tubulin polymerization. MAP rich tubulin can polymerize efficiently at 1-2 mg/ml.

Storage and Reconstitution

The recommended storage conditions for the lyophilized material is 4°C and <10% humidity. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C.

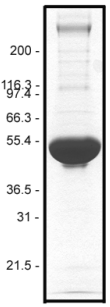
The protein should be reconstituted to 5 mg/ml with General Tubulin Buffer supplemented with 1 mM GTP. Snap freeze "experiment sized" aliquots in liquid nitrogen and store at -70°C. Reconstituted ML116 is stable for 6 months at -70°C. **Reconstituted ML116 MUST be snap frozen in liquid nitrogen prior to storage at-70°C, failure to do this will result in significant loss of activity.**

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. ML116 is determined to be composed of approximately 70% tubulin (55 kDa) and 30% MAPs.

Figure 1. ML116 Protein Purity Determination

A 40 µg sample of MAP-rich tubulin 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. The arrow head indicates the tubulin protein (55 kDa).



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Biological Activity Assay

The biological activity of ML116 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 2). Under the experimental conditions defined below a 2 mg/ml tubulin solution in General Tubulin Buffer buffer and 1 mM GTP should achieve an OD340 nm absorption reading between 0.3 - 0.5 in 20 min at 37° C. The assay volume is 180 µl and assumes a spectrophotometer pathlength of 0.8 cm. 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 well plate (Corning Cat. # 3696) for optimal polymerization signal in this assay.

Reagents

1. MAP-rich tubulin protein (Cat. # ML116)
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

Equipment

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

Method

1. Aliquot 5 ml of ice cold General Tubulin Buffer into a tube, add 50 µl of 100 mM GTP stock to give a final buffer composition of General Tubulin Buffer containing 1 mM GTP. Keep this on ice and use within 2-4 h.
2. Resuspend a 1 mg vial of ML116 with 0.5 ml of the ice cold buffer to give a 2 mg/ml final protein concentration.
3. Leave the protein on ice for 2-3 min to soften the tubulin protein pellet.
4. The vial of protein should then be mixed well with a pipette to make sure that the protein has resuspended evenly.
5. MAP-rich tubulin is a labile protein and should be used immediately after resuspension. Keep protein on ice prior to beginning the polymerization reaction.
6. For a standard 96 well plate assay you should transfer 180 µl of the resuspended ML116 (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.
7. 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.
8. Note: Temperature is an extremely important parameter for tubulin polymerization. MAP-rich tubulin is less sensitive to temperature than pure tubulin, however, 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 more noticeable nucleation phase (Phase I, Figure 2).
9. Figure 2 shows the results of polymerizing ML116 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 MAP-rich tubulin concentration to 1 mg/ml. These conditions will result in a more pronounced nucleation phase and a shallower polymerization curve. In this case, efficient polymerization is achieved by addition of an enhancer such as taxol (5 - 10 µM final concentration).

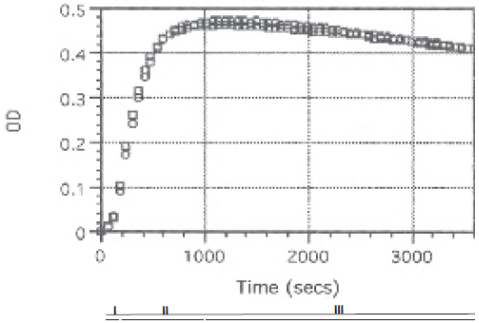

Figure 2: Tubulin Polymerization Assay. Tubulin polymerization was carried out as indicated in the Method section. Briefly, the polymerization reaction contains 180 µl of 2 mg/ml tubulin in 80 mM PIPES pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$, 1 mM GTP. Polymerization was started by incubation at 37°C and followed by absorption readings at 340 nm. Under these conditions polymerization reached a maximal OD340 between 0.3 - 0.5 within 20 min. In this experimental set up (180 µl 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 100% 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

- Substrate for anti-tubulin drug discovery.
- Substrate for tubulin MAP interaction studies.

References

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

Product Citations/Related Products

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