V. 1.0

Microtubule Associated Protein Fraction (Bovine brain)
Cat. # MAPF

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

Material

A microtubule associated protein (MAP) fraction has been isolated from bovine brain by temperature induced tubulin polymerization followed by ionic exchange chromatography over a phosphocellulose matrix and salt elution (1, 2). The MAP fraction protein is supplied as a white lyophilized powder

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 1 mg/ml by the addition of 100 µl of Milli-Q water. The protein will be in the following buffer: 10 mM PIPES pH 7.5, 0.3 mM EGTA, and 3% (w/v) sucrose. In order to maintain high biological activity of the protein fraction, it is recommended that the protein solution be aliquoted into "experiment sized" amounts, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for 6 months if stored at -70° C. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for 1 year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gel. MAP2 constitutes 70% of the total protein (see Figure 1).

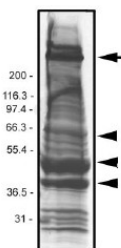

Figure 1. Microtubule Associated Protein Fraction Purity Determination. A 10 µg sample of MAP fraction protein was separated by electrophoresis in a 12% SDS-PAGE system, and stained with Coomassie Blue. Arrow indicates MAP1 and MAP2 (approx. 250 kDa), arrowheads indicate tau isoforms. Protein quantitation was performed using the Precision Red™ Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of the MAP fraction can be determined by the ability of 0.1 mg/ml MAP fraction to enhance the polymerization rate (Vmax) of purified bovine brain tubulin *in vitro*. Stringent quality control ensures that the MAP fraction protein will stimulate tubulin polymerization approximately five fold when compared to tubulin polymerization without MAP fraction.

Reagents

1. Microtubule associated protein fraction (100 µg, Cat. # MAPF)
2. Bovine brain tubulin (Cat. # TL238)
3. General Tubulin Buffer (80 mM PIPES pH 7.0, 0.5 mM EDTA, 2 mM $MgCl_2$. Cat. # BST01)
4. 100 mM GTP solution (Cat. BST06)
5. 5% glycerol in General Tubulin Buffer

Equipment

1. Temperature regulated spectrophotometer capable of measuring absorbance at 340 nm
2. Half area 96 well microtiter plate (Corning Costar, Cat. # 3697)
3. Multi-channel pipettor

Method

1. Warm the 96 well half area plate and the spectrophotometer to 37°C prior to resuspending the lyophilized bovine brain tubulin or MAP fraction.
2. Resuspend the bovine brain tubulin to 2 mg/ml in General Tubulin Buffer plus 5% glycerol and
3. 1 mM GTP. NOTE: GTP should be added fresh from a 100 mM stock just prior to use.
4. Leave the protein on ice for 5-10 minutes to soften the tubulin protein pellet.
5. The vial of protein should then be mixed well with a pipette to make sure that the protein is thoroughly resuspended. Tubulin is a labile protein and should be used immediately after resuspension. Keep tubulin on ice prior to beginning the polymerization reaction.
6. Resuspend the MAP fraction protein to 1 mg/ml. Do not vortex as this will denature the protein. Keep on ice.
7. Add 10 µl of the resuspended MAP fraction into duplicate wells of the half are plate, this will resultin a 0.1 mg/ml final concentration in the polymerization reactions. Prepare negative control polymerization reactions by adding 5 µl of General Tubulin Buffer to duplicate wells.
8. Immediately transfer 90 µl of the tubulin protein into all four wells using a multi-channel pipettor.
9. Measure tubulin polymerization by taking readings once

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every 30 seconds at 340 nm and 37°C. It is not necessary to designate a "BLANK" well. All wells can be blanked individually at the start of the readings.

10. 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 the polymerization reaction.
11. It is recommended to read the polymerization reaction for 45 minutes to 1 hour.
12. Under these conditions, a 2 mg/ml solution of bovine brain tubulin (Cat. # TL238) will reach an OD_{340} between 0.18 - 0.25 after 1 hour in the presence of 0.1 mg/ml MAP fraction protein and approximately 0.08 in the absence of MAP fraction protein. The polymerization rate (V_{max}) of bovine brain tubulin is enhanced approximately 5 fold in the presence of MAP fraction protein. Typical results are shown in Figure 2.0

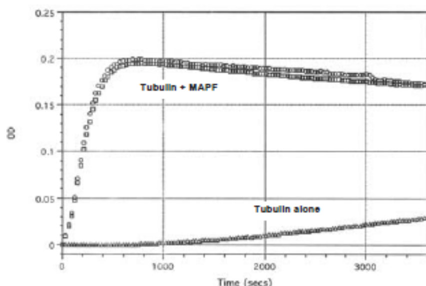

Figure 2. Tubulin Polymerization in the Presence and Absence of MAP Fraction Protein. Tubulin polymerization reactions were carried out as described in the Method. All assays show 2 mg/ml of pure bovine brain tubulin (Cat. # TL238) being polymerized in the presence and absence of 0.1 mg/ml MAP fraction protein. MAP fraction protein is shown to enhance the polymerization rate (Vmax) of tubulin approximately five fold.

Product Uses

- Positive control for the study of microtubule binding proteins
- Investigation of the effect of MAPs on microtubule dynamics
- Substrate for many protein kinases

References

1. Vallee, R.B. 1982. *J. Cell Biol.* 92(2):435-442
2. Kuznetsov S.A., et al. 1981. *FEBs Lett.* 135(2): 241-244

Product Citations/Related Products

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