V. 2.0

Tau Proteins
Source: Bovine brain
Cat. # TA01

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

Material

Tau proteins have been isolated from MAP-rich bovine brain tubulin by ion exchange chromatography over a phosphocellulose matrix. Tau proteins have molecular weights between 40-70 kDa and are know to be post-translationally modified. Tau protein is supplied as a white lyophilized powder.

Storage and Reconstitution

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 1 mg/ml with the addition of 50 µl of cold Milli-Q water. When reconstituted, the protein will be in the following buffer: 40 mM PIPES pH 7.5, 0.8 mM EDTA, 0.8 mM EGTA, 5% mannitol, 1% dextran and 10 mM NaCl. In order to maintain high biological activity of the protein it is strongly 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 six months if stored at -70°C. **The protein must not be exposed to repeated freeze-thaw cycles.** The lyophilized protein is stable at 4°C desiccated (<10% humidity) for six months.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gradient gel. Tau protein was determined to be >90% pure (see Figure 1). 1% of contaminants are high molecular weight MAPs and 9% are other MAPs and non-MAP proteins.

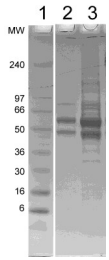

Figure 1. Tau Protein Purity Determination. Samples of Tau protein (molecular weight approx. 40-70 kDa) were separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Lane 1 = Molecular Weight Markers (SeeBlue Invitrogen), Lane 2 = 2 µg of TA01, Lane 3 = 10 µg of TA01.

Biological Activity Assay

The biological activity of Tau protein can be determined by its ability to enhance the polymerization of tubulin. Stringent quality control ensures that tubulin polymerization will be enhanced 10 fold in the presence of 100 µg/ml Tau protein.

Reagents

1. Tau proteins (Cat. # TA01)
2. Tubulin protein (Cat. # TL238)
3. GTP stock 100 mM (Cat. # BST06)
4. General Tubulin Buffer (Cat. # BST01: 80 mM PIPES pH 6.9, 0.5 mM EDTA, 2 mM $MgCl_2$)

Equipment

1. Temperature regulated spectrophotometer (wavelength set at 340 nm)
2. Half area 96 well plate (180 µl volume wells) (Corning Costar, Cat # 3696)

Method

1. Warm the 96 well half area plate and the spectrophotometer to 37°C prior to resuspending the tubulin protein and Tau protein.
2. Resuspend Tau protein as described under Storage and Reconstitution. Keep on ice.
3. Resuspend tubulin to 4 mg/ml in General Tubulin Buffer plus 4 mM $MgCl_2$, 0.5 mM EGTA and 1 mM GTP. NOTE: GTP should be added fresh from a 100 mM stock just prior to use.
4. Leave tubulin on ice for 5-10 min to dissolve the tubulin protein powder. The vial of protein should then be mixed well with a pipette to make sure that the protein is thoroughly resuspended. Keep tubulin on ice prior to beginning the polymerization reaction.
5. Pipette 10 µl of General Tubulin Buffer to two wells (control wells) and 10 µl of Tau protein to remaining wells.
6. Immediately pipette 90 µl of tubulin protein to the four wells.
7. Measure tubulin polymerization by taking readings once every 60 s for 60 min at 340 nm and 37°C. Note: Temperature is an extremely important parameter for tubulin polymerization, A temperature cooler than 37°C will significantly decrease the rate and final OD reading of the polymerization reaction.
8. It is recommended to read the polymerization reaction for 30 min to 1 h.
9. Under these conditions, a 4 mg/ml solution of bovine brain tubulin will reach an OD_{340} between 0.10 - 0.15 after 30 min in the presence of 100 µg/ml Tau protein and approximately 0.02 in the absence of Tau proteins. The polymerization

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rate (V_{max}) of bovine brain tubulin is enhanced over 10 fold in the presence of Tau proteins.

10. A typical polymerization curve is shown in Figure 2.

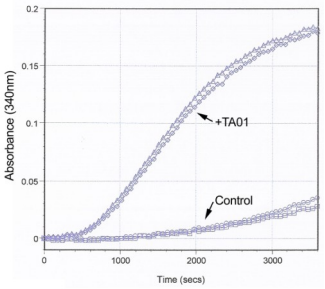

Figure 2. Tubulin polymerization enhancement by Tau proteins. Bovine brain tubulin was polymerized with and without Tau protein as described in the method section. In the presence of 100 µg/ml Tau protein tubulin polymerization was enhanced 10 fold compared to tubulin alone.

Product Uses

- Control protein for neuronal plaque and Alzheimers research.
- Control protein for studying microtubule dynamics.
- Substrate for protein kinases.

Product Citations/Related Products

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