V. 3.0

Paclitaxel (Reagent Grade)
Other Name Taxol™
Cat. # TXD01

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

Material
Paclitaxel ($C_{47}H_{51}NO_{14}$) has a molecular weight of 853.9 g/mol and is supplied as a lyophilized white powder.

Storage and Reconstitution
Lyophilized paclitaxel is stable at 4°C desiccated (<10% humidity) for 1 year. Each tube of paclitaxel should be resuspended in 100 µl of anhydrous Dimethyl sulfoxide (DMSO), (99.9+%) to give a 2 mM stock solution that is stable for 6 months if stored at -20°C. Ethanol can be used as an alternative solvent to DMSO. Methanol should be avoided as the paclitaxel will undergo transesterification in this solvent.

Health Hazards
Paclitaxel is toxic (LD50: 32.5 mg/kg: rat [intraperitoneal]) and is a potential carcinogen. Primary route of entry for paclitaxel powder is inhalation. After reconstitution in DMSO the primary route of entry is skin absorption. Always wear a mask and chemically impervious gloves when handling paclitaxel. Work in a hood when handling powder. Disposal of paclitaxel must be in accordance with federal, state, and local regulations.

Purity
Paclitaxel is determined chromatographically to be 99.1% pure.

Uses
Paclitaxel is used in vitro to stabilize microtubules. The compound should be added to microtubules to a final concentration of 10-20 µM (i.e. a 1:200 - 1:100 dilution of the 2 mM stock solution). Paclitaxel stabilized microtubules should be stable at room temperature for 3 days.

Activity Assay
Biological activity of the Paclitaxel stock is determined by the ability of 10 µM paclitaxel to enhance the polymerization rate (Vmax) of bovine brain tubulin in vitro.

Reagents
1. Bovine brain tubulin (lyophilized protein) (Cat. # TL238)
2. General Tubulin Buffer (Cat. # BST01; 80 mM PIPES buffer pH 6.9, 0.5 mM EGTA, 2 mM $MgCl_2$)
3. 100 mM GTP solution (Cat. # BST06)
4. 5% glycerol in General Tubulin Buffer
5. Paclitaxel stock 2 mM in DMSO (Cat. # TXD01)

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

Method
1. Warm the 96 well 1/2 area plate and the spectrophotometer to 37°C prior to resuspending the lyophilized bovine brain tubulin (Cat. # TL238).
2. Resuspend the bovine brain tubulin to 1 mg/ml in General Tubulin Buffer plus 5% glycerol and 1 mM GTP. NOTE: GTP should be added fresh from a 100 mM stock just prior to use.
3. Leave the protein on ice for 5-10 minutes 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 is thoroughly resuspended.
5. Tubulin is a labile protein and should be used immediately after resuspension. Keep tubulin on ice prior to beginning the polymerization reaction.
6. In a separate eppendorf tube, dilute 10 µl of the paclitaxel stock with 90 µl of room temperature General Tubulin Buffer.
7. Add 5 µl of the diluted Paclitaxel stock to duplicate wells, this will give 10 µM paclitaxel in the final polymerization reactions. Prepare negative control polymerization reactions by adding 5 µl of General Tubulin Buffer to duplicate wells.
8. Immediately transfer 95 µl of the tubulin protein into all four wells using a multi-channel pipettor.
9. Measure tubulin polymerization by taking readings once 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 lower 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 1 mg/ml solution of bovine brain tubulin (Cat. # TL238) will reach an OD_{340} between 0.06 - 0.1 after 1 hour in the presence of 10 µM paclitaxel and approximately 0.02 in the absence of paclitaxel. The polymerization rate (V_{max}) of bovine brain tubulin is enhanced approximately six fold in the presence of paclitaxel.

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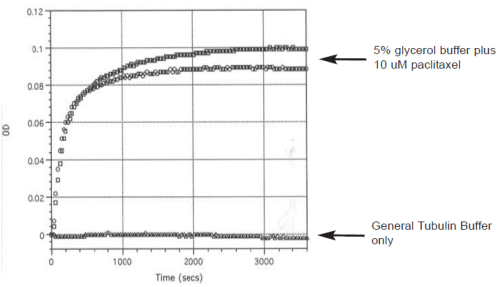

Figure 1: Tubulin Polymerization in the presence and absence of Paclitaxel
Polymerization reactions were carried out as described in the Method. All assays show 1 mg/ml of pure bovine tubulin (Cat. # TL238) being polymerized in the presence and absence of 10 µM paclitaxel. Assays are shown in duplicate. Paclitaxel is shown to enhance the polymerization rate (V_{max}) of the tubulin approximately six fold.

Formation of Paclitaxel Stabilized Microtubules
Tubulin protein consists of a heterodimer of one alpha and one beta tubulin polypeptide. It polymerizes *in vivo* and *in vitro* to form microtubules (MTs). These are highly dynamic structures that continually undergo polymerization and depolymerization. In the cell, MT dynamics are regulated by Microtubule-Associated Proteins (MAPs) which control MT dependent events such as mitosis and intracellular transport. *In vitro* MT polymerization and dynamics are highly dependent upon reaction conditions such as temperature, glycerol concentration, and Mg^{2+} concentration. For example, tubulin will polymerize at 37°C and depolymerize at 4°C. Paclitaxel binds to an internal region on MTs and stabilizes them against depolymerization both *in vivo* and *in vitro*.

Experimentally, MTs are commonly used as substrates in the study of a wide variety of MAPs. It is therefore convenient to use paclitaxel stabilized microtubules in these assays as microtubule dynamics may complicate interpretation of data. The method described on the next page outlines how to form paclitaxel stabilized MTs.

Reagents
1. 1 mg Bovine brain tubulin (lyophilized protein) (Cat. # TL238). Reconstitute 1 mg of lyophilized tubulin to 5 mg/ml in 200 µl of ice cold General Tubulin Buffer plus 10% glycerol and 1 mM fresh GTP and aliquot into 20 µl experiment sized volumes. Snap freeze the aliquots in liquid nitrogen and store at -70°C.
2. General Tubulin Buffer (Cat. # BST01; 80 mM PIPES buffer pH 6.9, 0.5 mM EGTA, 2 mM MgCl2)
3. 100 mM GTP solution (Cat. # BST06)
4. 10% glycerol in General Tubulin Buffer
5. Paclitaxel stock 2 mM in DMSO (Cat.# TXD01)

Equipment
1. Water bath at 37°C

Method
1. Warm 200 µl of General Tubulin Buffer plus 10% glycerol to 37°C. This will be used later.
2. Thaw out a 20 µl aliquot of 5 mg/ml tubulin and incubate at 37°C for 20 minutes to allow MT formation.
3. After the tubulin has incubated for 15 minutes, remove the 200 µl of General Tubulin Buffer plus 10% glycerol from 37°C and add 2 µl of the 2 mM paclitaxel stock to give a 20 µM final paclitaxel solution. Leave this at room temperature.
4. After the tubulin has incubated for 20 minutes remove it from the water bath and IMMEDIATELY add the 200 µl of paclitaxel supplemented General Tubulin Buffer plus 10% glycerol. Mix thoroughly but gently and leave the MTs at room temperature.

You now have a population of stable MTs that are between 5-10 µm in length and that are at an approximate concentration of 2.2 x 10^{11}/ ml. This is equivalent to 4.5 µM tubulin heterodimer or 0.36 nM microtubules. The MTs should be stable at room temperature for 3 days. NOTE: some alternative methods of stable MT formation use paclitaxel in the polymerization reaction. This has been found to enhance the formation of aberrant MTs and is NOT RECOMMENDED.

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