V. 1.1

General Tubulin Buffer
Cat. # BST01

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

Material
General Tubulin Buffer is supplied as a white lyophilized powder.

Storage and Reconstitution
Lyophilized General Tubulin Buffer is stable at 4°C desiccated (<10% humidity) for 1 year. Each bottle of General Tubulin Buffer should be resuspended in 100 ml of de-ionized water to give a 1X strength buffer: 80 mM PIPES, 2 mM $MgCl_2$, 0.5 mM EGTA pH 7.0.

Product Uses
Used as an in vitro buffer for tubulin proteins from a wide range of species. This is the buffer of choice when working with biologically active tubulin proteins. The buffer should be supplemented with 1 mM GTP (Cat. # BST06) to create an optimal tubulin buffer. Magnesium ions and GTP are required for tubulin conformation. GTP is also required for the polymerization process and is hydrolyzed during tubulin polymerization. EGTA is a chelator of calcium which is a potent inhibitor of tubulin polymerization.
Glycerol is often added to a final concentration of 5-10% to enhance polymerization; however, glycerol is not necessary for the maintenance of biologically active tubulin.

Biological Activity Assay
General Tubulin Buffer is tested for its ability to support tubulin polymerization in vitro. Under the conditions of the polymerization assay given below, General Tubulin Buffer should support the polymerization of tubulin to an OD_{340} of 0.16 OD units per mg of tubulin.

Reagents
1. Porcine brain tubulin (lyophilized protein) (Cat. # T240)
2. General Tubulin Buffer (Cat. # BST01)
3. 100 mM GTP solution (Cat. # BST06)
4. 5% glycerol in General Tubulin Buffer

Equipment
1. Temperature-regulated spectrophotometer (wavelength 340 nm)
2. Half area 96 well plate (180 ul 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 porcine brain tubulin (Cat. # T240).
2. Resuspend the porcine brain tubulin to 5 mg/ml in ice-cold 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. Immediately transfer 100 ul of the tubulin protein into duplicate wells of a half area 96 well plate using a multi-channel pipettor.
7. 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. **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.
8. It is recommended to read the polymerization reaction for 45 minutes to 1 hour.
9. Under these conditions, a 5 mg/ml solution of porcine brain tubulin (Cat. # T240) will reach an OD340 between 0.75 - 1.0 after 1 hour (see Fig. 1).

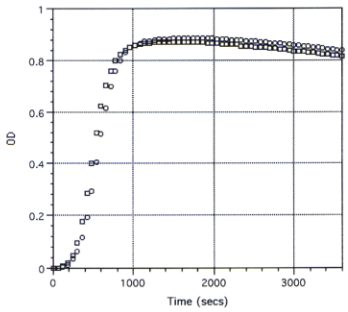

Figure 1: Tubulin Polymerization in the presence of General Tubulin Buffer. Polymerization reactions were carried out as described in the Method. All assays show 5 mg/ml of pure porcine tubulin (Cat. # T240) being polymerized in the presence of 1X General Tubulin Buffer plus 1 mM GTP and 5% glycerol. Assays are shown in duplicate.

Product Citations / Related Products
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