

## Tubulin Glycerol Buffer (Cushion Buffer)

Cat. # BST05

Upon arrival store at 4°C (desiccated)

See datasheet for storage after reconstitution

### Material

Tubulin Glycerol Buffer (Cushion Buffer) is supplied as a clear liquid.

### Storage and Reconstitution

The buffer is stable at 4°C for 1 year.

Buffer composition: 1X strength; 80 mM PIPES, 2 mM MgCl<sub>2</sub>, 0.5 mM EGTA, 60% glycerol pH 6.9.

### Uses

Tubulin Glycerol Buffer can be used to supplement General Tubulin Buffer (BST01-010) to create a final glycerol concentration of 5-10%. Glycerol is often added to a final concentration of 5-10% to enhance polymerization (see Figure 1), however, glycerol is not necessary for the maintenance of biologically active tubulin.

The buffer should be supplemented with 1 mM GTP (Cat. # BST06-001) to create an optimal tubulin buffer. GTP is hydrolysed during tubulin polymerization and is required for the polymerization process. Magnesium ions and GTP are required for tubulin conformation, EGTA is a chelator of calcium which is a potent inhibitor of tubulin polymerization.

Tubulin Glycerol Buffer (Cushion Buffer) can also be used undiluted as a 60% glycerol cushion. Cushion Buffer is used to separate microtubules from non-polymerized tubulin. The cushion also provides a method of separating microtubules and associated proteins (MAPs) from unpolymerized or denatured tubulin and non-MAP proteins. Microtubules are layered onto a 5x volume of Cushion Buffer and centrifuged at 100,000g for 30 minutes. The microtubules will pellet through the cushion and leave any unpolymerized tubulin and non-MAP proteins at the cushion buffer interface. The cushion can be gently removed and the microtubule pellet can be resuspended and analysed as required. It is important to note that the Cushion Buffer should be supplemented with any reagents that are necessary for tubulin / microtubule stability e.g. GTP (1mM), taxol (10 µM).

### Activity Assay

Tubulin Glycerol Buffer is tested for its ability to support tubulin polymerization *in vitro*. Under the conditions of the polymerization assay given below, Tubulin Glycerol Buffer should support the polymerization of tubulin to an OD<sub>340</sub> of 0.16 OD units per mg of tubulin.

### Reagents

1. Bovine brain tubulin (lyophilized protein) (Cat. # TL238)
2. General Tubulin Buffer (Cat. # BST01-010)
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 µl volume wells) (Corning Costar, Cat # 3696)

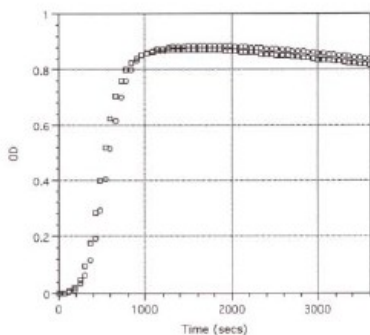
### Method

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 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 µl 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.
8. 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.
9. It is recommended to read the polymerization reaction for 45 minutes to 1 hour.

10. Under these conditions, a 5 mg/ml solution of bovine brain tubulin (Cat. # TL238) will reach an OD<sub>340</sub> between 0.75 - 1.0 after 1 hour (see Fig. 1).

#### Product Citations/Related Products

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**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 bovine tubulin (Cat. # TL238) being polymerized in the presence of 1X General Tubulin Buffer plus 1mM GTP and 5% glycerol. Assays are shown in duplicate.