



## CYCLING TUBULIN

### Materials:

- Lyophilized Tubulin (Cat. No. PUR-142001)
- Tubulin PEM Buffer (Cat. No. PUR-032002; 80 mM PIPES, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, pH = 6.8)
- Glycerol
- Glycerol Cushion (60% v/v glycerol in Tubulin PEM Buffer)
- Liquid Nitrogen

### Equipment:

- Ultracentrifuge and rotor (i.e. Beckman TL-100 Tabletop Ultracentrifuge and TLA-100 rotor)
- Water bath at 37°C

### Technical Notes:

- Avoid diluting tubulin beyond its critical concentration
- Work in a 37°C water bath when tubulin is polymerized, work in an ice bucket when tubulin is depolymerized
- Pre-warm/chill necessary buffers, centrifuges, and rotors as needed

### Protocol:

#### 1. Reconstitute Lyophilized Tubulin to 40 mg/ml (see respective protocol for details)

#### 2. Polymerize

- a) Add GTP to 1 mM
- b) Incubate on ice for 5 minutes
- c) Incubate in a 37°C water bath for 2 minutes
- d) Add 1/2 volume of pre-warmed glycerol
- e) Incubate in a 37°C water bath for 40 minutes

#### 3. Pellet Microtubules

- a) Layer polymerized microtubules over a pre-warmed glycerol cushion

**\*cushion should be roughly twice the volume of the polymerization reaction**

- b) Spin at 70,000 rpm for 30 minutes at 37°C in a pre-warmed ultracentrifuge and rotor
- c) Wash the supernatant/cushion interface three times with pre-warmed Tubulin PEM Buffer
- d) Pipet off remaining cushion
- e) Gently wash the microtubule pellet with pre-warmed Tubulin PEM Buffer

#### 4. Depolymerize

- a) Resuspend the microtubule pellet in a minimal amount of ice-cold Tubulin PEM Buffer

**\*recommended to use 1/2 volume of original polymerization reaction**

- b) Incubate on ice for 30 minutes with occasionally mixing by pipet



#### 5. Clarify

- a) Spin at 70,000 rpm for 15 minutes at 4°C in a pre-chilled ultracentrifuge and rotor
- b) Collect the supernatant on ice

**\*tubulin can be diluted as desired at this point**

#### 6. Store

- c) Flash freeze in liquid nitrogen and store at -80°C

**\*aliquot so as to avoid repeated freeze-thaw cycles**