



POLYMERIZING CYCLED TUBULIN™



Materials:

- Cycled Tubulin™ (Cat. No. PUR-032005)
- Tubulin PEM Buffer (Cat. No. PUR-032002; 80 mM PIPES, 1 mM EGTA, and 1 mM MgCl₂, pH 6.8)
- DTT
- GTP or GMPCPP
- Taxol (optional)

Equipment:

- Water bath at 37°C

Technical Notes:

- Avoid diluting tubulin beyond its critical concentration
- If stabilized microtubules are desired, either polymerize with GMPCPP or stabilize with Taxol
- Add Taxol stepwise to avoid precipitation
- Do not place polymerized microtubules on ice

Protocol:

1. Assemble the Polymerization Reaction

- a) Dilute Cycled Tubulin™ to 2 mg/ml with Tubulin PEM Buffer
- b) Add DTT and guanosine (GTP or GMPCPP) to 1 mM each
- c) Incubate on ice for 5 minutes

2. Polymerize

- a) Incubate in a 37°C water bath for 1 hour

3. Stabilize with Taxol (optional)

- a) Add 1/10 volume Taxol at 2 µM, incubate in a 37°C water bath for 10 minutes
- b) Add 1/10 volume Taxol at 20 µM, incubate in a 37°C water bath for 10 minutes
- c) Add 1/10 volume Taxol at 200 µM, incubate in a 37°C water bath for 15 minutes

***include Taxol to 200 µM in subsequent buffers**