



GENERATING FLUORESCENT MICROTUBULES



Materials:

- Unlabeled tubulin: Cycled Tubulin™ (Cat. No. PUR-032005) or Lyophilized Tubulin (Cat. No. PUR-142001)
- Labeled tubulin: Alexa Fluor® 488 (Cat. No. PUR-048805), 594 (Cat. No. PUR-059405), or 647 (Cat. No. PUR-064705)
- 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:

- Protect from light
- Adjust the labeled:unlabeled tubulin ratio as needed
- 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) Combine labeled tubulin at 0.2 mg/ml and unlabeled tubulin at 1.8 mg/ml in 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 uM, incubate in a 37°C water bath for 10 minutes
- b) Add 1/10 volume Taxol at 20 uM, incubate in a 37°C water bath for 10 minutes
- c) Add 1/10 volume Taxol at 200 uM, incubate in a 37°C water bath for 15 minutes

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