Tubulin polymerizing protocol.

Polymerized tubulin forms microtubules which can be visualized on a high-end microscope using DIC or, if fluorescently labeled tubulin is used, on a fluorescence capable instrument.

Buffers

PEM80	in 250mL
80 mM Pipes (Sigma P1851)	6.048 g
1 mM EGTA (Sigma E-4378)	95.1 mg
4 mM $MgCl_2$ (Mallinckrodt H590)	204.1 µL of 4.9 M stock
pH adjust to 6.9 with KOH	

PEM104	in 100mL
103.6 mM Pipes	3.133 g
1.3 mM EGTA	49.452 mg
6.3 mM $MgCl_2$	128.57 µL of 4.9 M stock
pH adjust to 6.9 with KOH	

STAB
34.1 µL PEM80
5 µL 10 mM GTP (Cytoskeleton BST06)
4.7 µL 65g/L NaN_3 (Sigma S-8032)
1.2 µL 10mM Taxol (Cytoskeleton TXD01)
5.0 µL DMSO (Sigma D-5879)

Protocol

1. Take the tubulin and spin for approximately 30 minutes at 4°C
2. Combine:
   15.2 µL PEM104
   2.0 µL 10 mM GTP
   to make a PEM/GTP solution
3. Combine:
   15.2 µL of the PEM/GTP soln
   2.2 µL of DMSO
   and vortex before adding
   4.8 µL of 10mg/mL tubulin (Cytoskeleton T237 – **non** fluorescent labeled)
   use Cytoskeleton T331 Rhodamine labeled for fluorescent microtubules
   to make the "TUB" solution
4. Place the TUB soln in a water bath at 37°C for 30 minutes
5. Remove TUB from the water bath and add 2 µL of STAB
6. Store the newly formed microtubules at room temperature