

Protocol 3: Preparation of GMPCPP-stabilized microtubule seeds.

This procedure will take ~ 1 hr and the resulting microtubule seeds are stable for 1-2 days at room temperature.

3.1. Mix on ice:

- 10 μ L unlabeled tubulin (100 μ M) in BRB-80 buffer (80 mM Pipes, 1 mM EGTA, 4 mM $MgCl_2$, pH 6.9 with KOH; supplement with 1-2 mM DTT using fresh aliquot for each experiment).
- 2.6 μ L digoxigenin-labeled tubulin. Adjust volume depending on preparation, such that the final ratio of labeled to unlabeled tubulin is ~ 1:10. Mix well by pipetting.
- 1.4 μ L 10 mM GMPCPP (final concentration 1 mM)

3.2. Incubate 15 min at 35°C, the seeds will grow 2-3 μ m long. Adjust time if different microtubule length is desired.

3.3. Add 35 μ L BRB-80 (prewarmed to 35°C), mix by pipetting, and centrifuge for 15 min at 25,000 G to pellet the seeds at room temperature.

3.4. Discard supernatant, wash the pellet by gently adding and removing 50 μ L of warm BRB-80.

3.5. Resuspend pellet well in 25 μ L BRB-80.