

Preparation of Segmented and Polarity Marked Microtubules

Segmented and polarity-marked microtubules are very useful for many different types of *in vitro* assays. Segmented microtubules are microtubules with a bright seed and dim elongated segments on both ends. Polarity marked microtubules are microtubules with a bright seed and a dim elongated segment only on one end -- the plus end. Selective elongation of one end is achieved by inclusion of NEM-treated tubulin, a competitive inhibitor of minus end polymerization. More complex microtubule substrates such as GDP microtubule lattices capped with segments of GMPCPP tubulin can also be prepared by playing around with *in vitro* polymerization conditions. Also, segmented and polarity marked microtubules can be made from different color tubulins instead of different intensity of a single color tubulin as described here.

Note that the precise ratios of labeled to unlabeled tubulin described here may need to be adjusted depending on the labeled tubulin prep. Described here is what has worked well for us using tetramethylrhodamine NHS ester-labeled tubulin (stoichiometry of labeling ~ 1.4).

I. Solutions & Supplies

BRB80 (1X): 80 mM PIPES, 1 mM MgCl₂, 1 mM EGTA, pH 6.8 with KOH (generally made as a 5X stock and stored at 4°C)

100 mM GTP

100 mM GMPCPP

Taxol: 10 mM stock; 200 μ M, 20 μ M and 2 μ M dilution all in anhydrous DMSO; sold under tradename "Paclitaxel" by [Sigma](#)

Bright GMPCPP Seed Mix (2 mg/ml; 1 part [rhodamine tubulin](#) to 2 parts [unlabeled tubulin](#); prepared and stored at -80°C as described above)

NEM GTP-Tubulin (prepared by treating [recycled tubulin](#) (~ 5 -15 mg/ml) in BRB80 + 0.5 mM GTP with 1 mM NEM (N-ethylmaleimide; from a fresh 50 mM stock in water prepared just before use) for 10' at 0°C, quenching the NEM with 8 mM β -mercaptoethanol for 10' at 0°C, freezing aliquots in liquid nitrogen and storing at -80°C.

NEM CPP-Tubulin (prepared by treating [recycled tubulin](#) (~5-15 mg/ml) in BRB80 + 0.5 mM GMPCPP with 1 mM NEM (freshly prepared as a 50 mM stock in water) for 10' at 0iC, quenching NEM with 8 mM b-mercaptoethanol for 10' at 0iC, freezing aliquots in liquid nitrogen and storing at -80iC.

II. Segmented Taxol Microtubules

1. Polymerize bright GMPCPP seed mix at 37iC for 15'-30'.
2. On ice prepare the dim elongation mix consisting of 15 μ M tubulin (1 part [rhodamine tubulin](#) to 10 parts [unlabeled tubulin](#)) in 1X BRB80, 1 mM DTT, 1 mM GTP. Incubate at 0iC for 5' and (optionally) clarify at 90K for 5' at 2iC in a TLA100 rotor.
3. Incubate dim elongation mix at 37iC for 1'. Add 1/10-1/20 volume of GMPCPP seeds and mix gently. Incubate at 37iC for 20'. GMPCPP seeds are cold-labile and will depolymerize on ice. Therefore, only add the seeds after the elongation mix has warmed up.
4. [Add taxol stepwise](#) to 20 μ M.
5. The segmented taxol-stabilized microtubules can be pelleted over a glycerol cushion and resuspended, or used directly after dilution. All dilutions of taxol-stabilized MTs should be done into buffers containing 10-20 μ M taxol.

III. Polarity Marked Taxol Microtubules

1. Polymerize bright GMPCPP seed mix at 37iC for 15'-30'.
2. On ice prepare the dim polar elongation mix consisting of 15 μ M tubulin (1 part [rhodamine tubulin](#) to 10 parts [unlabeled tubulin](#)) and 12 μ M [NEM GTP-tubulin](#) in 1X BRB80, 1 mM DTT, 1 mM GTP. Incubate at 0iC for 5' and (optionally) clarify at 90K for 5' at 2iC in a TLA100 rotor.
3. Incubate dim polar elongation mix at 37iC for 1'. Add 1/10-1/20

volume of GMPCPP seeds and mix gently. Incubate at 37°C for 20'.

4. [Add taxol stepwise](#) to 20 μ M.

5. The polarity-marked taxol-stabilized microtubules can be pelleted over a glycerol cushion and resuspended, or used directly after dilution. All dilutions of taxol-stabilized MTs should be done into buffers containing 10-20 μ M taxol.

IV. Segmented GMPCPP Microtubules

1. Prepare dim GMPCPP Elongation Mix: 10 μ M (1 mg/ml) 1:9 [rhodamine labeled](#): [unlabeled tubulin](#) in 1X BRB80, 1 mM DTT, 0.5 mM GMPCPP. Incubate on ice for 5'-10', spin 90K 5' in TLA100 at 2°C, freeze in liquid nitrogen in 10 μ l aliquots (or use fresh).

2. Thaw GMPCPP bright seed mix by adding 9 vol of warm (37°C) BRB80 + 1 mM DTT (resulting in 2 μ M tubulin final) and incubate at 37°C for 30'-45'.

3. Thaw CPP elongation mix and store on ice. Dilute as follows on ice: 17 μ l BRB80 + 1 mM DTT 3 μ l CPP elongation mix (This results in elongation of \sim 1.5 μ M CPP-tubulin)

4. Incubate diluted CPP elongation mix at 37°C for 20 sec before adding 2 μ l of polymerized bright CPP seeds.

5. Incubate at 37°C for 1-2 hours. Pellet and resuspend or use directly.

V. Polarity Marked GMPCPP Microtubules

1. Prepare dim GMPCPP polar elongation mix: 10 μ M (1 mg/ml) 1:9 [rhodamine labeled](#): [unlabeled tubulin](#) and 8 μ M [NEM CPP-Tubulin](#) in 1X BRB80, 1 mM DTT, 0.5 mM GMPCPP. Incubate on ice for 5'-10', spin 90K 5' in TLA100 at 2°C, freeze in liquid nitrogen in 10 μ l aliquots (or use fresh).

2. Thaw GMPCPP bright seed mix by adding 9 vol of warm (37°C)

BRB80 + 1 mM DTT (2 μ M tubulin final) and incubate at 37°C for 30'-45'.

3. Thaw CPP polar elongation mix and store on ice. Dilute as follows on ice: 17 μ l BRB80 + 1 mM DTT 3 μ l CPP Polar Elongation Mix (This results in elongation of \sim 1.5 μ M CPP-tubulin)

4. Incubate diluted CPP polar elongation mix at 37°C for 20 sec before adding 2 μ l of polymerized bright CPP seeds.

5. Incubate at 37°C for 1-2 hours. Pellet and resuspend or use directly.