

Protocol for GMPCPP-MT polymerization

Materials (Use KOH to pH, not NaOH!)

- BRB80: 80mM PIPES, 1mM MgCl₂, 1mM EGTA, pH ~6.8
- S.B with K⁺: 25mM PIPES, 25mM KCl, 2mM MgCl₂, 1mM EGTA, pH ~6.8
- 100mM GTP, pH ~7 (aliquots in -80°C)
- 10mM GMPCPP, pH ~7 (100uL tubes in -80°C box, aliquots in -20°C, Na⁺ salt)
- Tubulin:
 - o Cytoskeleton (lyophilized powder, 250ug per tube)
 - o Tubulin prep (40uL of 6mg/mL, 240ug per tube in BRB80)

Preparing Tubulin

- 1) Warm heat block (add water), start cooling down rotor and ultracentrifuge
- 2) On ice, add 25uL cold BRB80 (10mg/mL) and 0.5uL GTP (2mM)
- 3) Cold spin: 100K rpm, 10 min, 4°C (keep supernatant)
- 4) *Polymerization*: add to prechilled glass vial, incubate at 37°C for >45 min
- 5) *Pellet MTs*:
 - a. 40K rpm, 20 min, 37°C
 - b. Discard supernatant, resuspend pellet in 25uL cold BRB80
- 6) *Depolymerization*: Pipet up/down well, leave on ice for 15 min
- 7) Measure conc. using Bradford (dilute ¼ first)

Polymerization with GMPCPP

- 1) *Nucleotide exchange*: Want 2-5uM tubulin and ~1mM GMPCPP final in BRB80
 - a. Add GMPCPP to tubulin, wait 15 min on ice
 - b. Pre-warm BRB80 in glass vial and add GMPCPP-tubulin directly
- 2) *Polymerization*: Incubate at 37°C for ~3 hours
- 3) Can leave overnight at R.T. if desired
- 4) *Spin down*:
 - a. Tabletop, 14K rpm for 15 min
 - b. Discard supernatant, wash pellet with 50uL warm S.B. (x2)
 - c. Resuspend pellet in 20uL warm S.B. (Cut tip and pipet up/down)
- 5) Check conc. using Bradford (depolymerize on ice for 15 min)

Making Complex

- 6) Add >2x excess kinesin, mix well (>10 min incubation at R.T.)
- 7) Add apyrase to digest ATP/ADP (add excess, wait longer than needed)
- 8) *Spin down*:
 - a. Tabletop, 14K for 15 min
 - b. Discard supernatant, wash pellet with 50uL warm S.B. (x2)
 - c. Resuspend pellet in warm S.B. (Cut tip and pipet up/down)

*From Daifei: "For ~37ug of MTs, resuspend pellet in 5uL, and add 0.7uL to grid"
- "Enough for ~7 grids" (at ~12uM), but usually 4-5uM is good from experience
- 4uL total: add water/S.B. + ATP/MgCl₂ + AlCl₃/NaF if needed