



RECONSTITUTING LYOPHILIZED TUBULIN



Materials:

- Lyophilized Tubulin (Cat. No. PUR-142001)
- EGTA
- MgCl₂
- Liquid Nitrogen

Equipment:

- Microcentrifuge at 4°C

Technical Notes:

- Avoid diluting tubulin beyond its critical concentration
- Work on ice with prechilled buffers and equipment

Protocol:

1. Resuspend

- a. Resuspend to 40 mg/ml in ice-cold Milli-Q water

***buffer conditions upon reconstitution with water will consist of 10 mM Sodium Phosphate, 0.5 mM MgCl₂, 0.1 mM GTP, and 0.25 M Trehalose, pH 7.0**

***1 mg tubulin = 3 mg powder**

- b. Add EGTA and MgCl₂ to 1 mM each

2. Clarify

- a. Clarify at 14,000 rpm for 1 minute at 4°C in a microcentrifuge
- b. Collect the supernatant on ice

***tubulin can be diluted, buffer exchanged or cycling as desired at this point**

3. Store

- a) Flash freeze in liquid nitrogen and store at -80°C

***aliquot so as to avoid repeated freeze-thaw cycles Add 1/10 volume Taxol at 2 μM, incubate in a 37°C water bath for 10 minutes**