CBG
Max Planck Institute
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Important Note: Most failures in tubulin prep happen because of lack of gaskets on the tubes. The liquid is sucked out by the vacuum and the centrifuge goes down. Make sure each tube has a gasket as you put the lid on. Also check the rotor. Keep and eye on your centrifuge every 20 mins throughout the run. AB is MES buffer. PB is Pipes buffer.

Procedure

1. Remove blood and excess meninges from the brain. Weigh the brains. Add equal volume of AB. Homogenixe 2X 30S and collect in a large beaker.
2. Decant into 6* 1L bottles and spin at 7K for 10 mins to clear out the major solids and also to remove the foam generated by homogenization.
3. Take supernatant and place in the 250-ml bottles. Weigh pairs of bottles roughly.
4. Spin 15K one hour. A tight pellet here increases the yield of the 1st sup.
5. Add ATP to 1.5mM, GTP to 0.5mM MgCl2 to 3mM. Then 1/2 volume 37C glycerol
6. Warm to 30C under a hot tap while swirling and then put in a 37C water bath for 1Hr.
7. Decant into type 19 bottles in the warm room and spin for 2 hours 19K. The microtubules will be in the **PELLET**!
8. Resuspend pellets in PB at 4C in the cold room. You can remove the pellets using a spatula. Resupend them using the motorized dounce homogenizer.
9. Check protein concentration is below 25mg/ml by Bradford. Use IgG as a standard.
10. Leave on ice for 40 mins.
11. Spin 30 mins type 45 4C. Keep the **SUPERNATANT**!
12. Add 37C glycerol to 50%, ATP to 1mM GTP to 0.5mM MgCl2 to 4mM. Bring to 37 and leave for 40 mins. Place in type 45 bottles in the warm room.
13. Spin 37C One hour type 45. **KEEP Pellet**. Resuspend the total volume in about 200mls of PB. Check tubulin concentration is below 35 mg/ml. Dounce. Leave on ice for 40mins. At this stage half of your protein is tubulin and half maps.
14. Centrifuge 45K Type 45 rotor. 30 mins 4C. Load on PC column.