Microtubule fractionation protocol

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| component | Notes | Stock concentration | Final concentration | volume |
| MOPS, pH 6.8 | Buffer range =6.5-7.9. Very little fluctuation of pH with temperature | 100 mM | 20 mM | 8 ml |
| NaCl | Keep it low to prevent dissociation of MAPs but not too low that it is too hypotonic during fractionation | 5M | 50 mM | 400 ul |
| NP-40 | Harsh enough to break PM but not nuclei | 10% | 0.1% | 400 ul |
| EGTA | Inhibitor | 500 mM | 2mM | 160 ul |
| MgCl2 |  | 1 M | 1 mM | 40 ul |
| Protease inhibitors | inhibitor |  | 1 tablet per 10 ml |  |
| NaVO4 | inhibitor | 200 mM | 2 mM | 400 ul |
| NaF | inhibitor | 500 mM | 2 mM | 160 ul |
| DTT | Reducing agent for tau solubility | 1M | 2 mM | 2 uL/ml |

* pH MOPS with NaOH
* buffer can be made ahead of time and frozen in single use aliquots at -20C
* Perform entire lysis procedure at RT with all solutions at RT. Cold temperatures rapidly destabilize microtubules.
* Add paclitaxel (4 ug/ml) final. Add these components fresh every time because of hydrolysis in aqueous solution. Paclitaxel kept as a stock in DMSO at -20C (4 mg/ml).
* Trypsinize cells (T75flask) with 2 ml 37C trypsin then dilute with 37C 20 ml PBS. Leave cells at 37C while counting. Count cells to normalize for cell number. Pellet equivalent number of cells at 300g for 5 min. Remove supernatant
* Add 1 ml lysis buffer to cells then transfer to eppendorf. Lyse by pulling 3 times through successive 21G and 26G syringes. This procedure takes about 3 min. Let the lysate sit for another 3 min.
* Spin at 1000g for 5 min to pellet nuclei
* Collect supernatant and spin at 18,000g for 10 min
  + Pellets mito and microtubules
* Collect supernatant (cytosolic + small membrane organelles and PM).
* Resolubilize pellet in 400 ul microtubule buffer (mito and microtubules)
  + Perform subsequent competition time course experiments as required.
  + Need about 80 ul of microtubule solution to visualize pellet after 18,000 g spins
* Measured fluorescence intensities of samples in corning black low volume 384 well plates. Loaded 18 uL of all samples except for cytoplasmic fraction (30 ul). Brought total volume of all samples to 30 ul
* Ran equivalent fractions (4 or 10 uL) of lysate on 10% gels for WB probing. Probed replicate blots for with mouse GFP (1:1000) or mouse tubulin (1:2000)
  + Reprobed GFP blots with Rabbit tau 1:1000