**Microtubules (MT) polymérization**

Tubulin >99% purity from Cytoskeleton Inc (TL238-C from cytoskeleton, Inc ; via *Universal Biologicals Cambridge)*

1mg Tubulin vials stored at -80°c. Resuspend 1 vial in 100l G-PEM and aliquote in 20l.

Snap freeze aliquotes (20ul, 10mg/ml) in liquid nitrogen, store at -80°.

**PEMD 1X**

**Stock for 1l for 500ml**

100mM Pipes pH6.8 0,5M 200ml 100ml

1mM EGTA 0,5M 2ml 1ml

1mM MgCl2 1M 1ml 0,5ml

1mM DTT 1M 1ml 0,5ml

**G-PEM (make fresh)**

80mM Pipes pH6.8

1mM EGTA

0,5mM MgCl2

1mM GTP (100M stock in -20°c)

**2X Tubulin polymerization buffer**

**Stock for 1ml**

160mM Pipes pH6.8 0,5M 320l

2mM EGTA 0,5M 4l

7mM MgCl2 1M 7l

12% DMSO 120l

H2O 549l

**Taxol (=Paclitaxel)**

Stock of taxol (#VWR 580555, diluted in DMSO at 5M, 50M, and 500M and stored at -20°c)

**« Quick and dirty » Tubulin polymerization protocol (Milligan, R. and Halpain, S. labs ):**

-1mg Tubulin Vial resuspended in 100l G-PEM

-spin 5min at max speed at 4°c (remove eventual aggregates)

-sup = tubulin 10mg/ml

96l 2X Tub Polym Buffer

+ 3l GTP 100mM (1,5mM final concentration)

+ 2l Taxol 50mM (0.5 mM final concentration)

+ 100l Tubulin 10mg/ml

incubate 22 min at 34°c

= 200l MT 5mg/ml (store at room temp, keep away from ice as cold induces MT depolymerization). These MTs can be stored 2-3 days on the bench.

**Tubulin polymerization protocol with 50****M taxol (from Benoit Roger) :**

We used this protocol in Denis Dacheux et al., “Human SAXO1 (FAM154A) Is a Microtubule-Stabilizing Protein Specific to Cilia and Related Structures,” Journal of Cell Science, February 11, 2015, jcs.155143, doi:10.1242/jcs.155143.

20l Tubulin 10mg/ml in G-PEM

+11l G-PEM (=31 l Tubulin)

🡪 +3l taxol 5M 🡪 incubate 5-10 min at 37°C

🡪 +3l taxol 50M 🡪 incubate 5-10 min at 37°C

🡪 +3l taxol 500M 🡪 incubate 15 min at 37°C

Total volume=40 l of microtubules (5 mg/ml tubulin) at 50M Taxol. Store at room temperature away from cold (otherwise MTs will depolymerize). These MTs can be stored 2-3 days on the bench.

**Co-sedimentation assay :**

- Overnight dialysis of your protein of interest against 1X PEMD

- Incubate increasing amounts of protein with fixed amount of MT (8M) (Start with protein amounts detectable using coomassie staining, 50 to 100ng of protein)

**Pipet MT using large orifice tips (or cut extremity of pipeting tips) as MT prep is very viscous**

4l MT 5mg/ml

+ your protein in 1x PEMD

+ PEMD 1X qsp 50l total volume

-incubate 1h at room temp (22°c)

-spin 15min at 100 000-190 000 g (30 000 rpm) in TLA100 rotor at 22°c

-collect supernatant + 12,5l 5X SDS loading buffer (see below for recipe)

-resuspend pellet in 62,5l 1X SDS loading buffer

load equal volume of each fraction (15-20l ) on SDS-PAGE.

**5X SDS loading buffer**

stock for 10ml for 50ml

0,25M Tris pH6,8 1M 2,5ml 12,5ml

10% SDS 1g 5g

50% Glycerol 100% 5ml 25ml

5mM EDTA 0,5M 100l 500l

0,1% Bromophenol Blue 10% 100l 500l

5% Mercapto Ethanol 100% 0,5ml 2,5ml