**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 1vial in 100 ml G-PEM and aliquote in 20 ml.

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**

80mM Pipes pH6.8

1mM EGTA

0,5mM MgCl2

1mM GTP (100 mM stock in -20°c)

**2X Tubulin polymerization buffer**

**Stock for 1ml**

160mM Pipes pH6.8 0,5M 320ml

2mM EGTA 0,5M 4ml

7mM MgCl2 1M 7ml

12% DMSO 120ml

H2O 549ml

**Taxol (=Paclitaxel)**

Stock of taxol (#VWR 580555, diluted in DMSO at different concentrations, stored at -20°c)

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

-1mg Tubulin Vial resuspended in 100ml G-PEM

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

-sup = tubulin 10mg/ml

96ml 2X Tub Polym Buffer

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

+ 2ml Taxol 50mM (0.5 mM final concentration)

+ 100ml Tubulin 10mg/ml

incubate 22 min at 34°c

= 200ml MT 5mg/ml (store at room temp, keep away from ice, cold induces MT depolymerization).

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

20ml Tubulin 10mg/ml in G-PEM

+11ml G-PEM (=31 ml Tubulin)

🡪 +3ml taxol 5mM 🡪 incubate 5-10 min at 37°C

🡪 +3ml taxol 50mM 🡪 incubate 5-10 min at 37°C

🡪 +3ml taxol 500mM 🡪 incubate 15 min at 37°C

Total volume=40 ml of microtubules (5 mg/ml tubulin) at 50mM Taxol. Store at room temperature away from cold.

**Co-sedimentation assay :**

Over-night dialysis of your protein of interest against 1X PEMD

Incubate increasing amounts of protein with fixed amount of MT (8mM) (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)

4ml MT 5mg/ml

+ your protein in 1x PEMD

+ PEMD 1X qsp 50ml 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 + 12,5ml 5X SDS loading buffer (see below for recipe)

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

load equal volume of each fraction (15-20 ml ) 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 ml 500 ml

0,1% Bromophenol Blue 10% 100 ml 500 ml

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