

July 10, 2012: Microtubules on Poly-L-lysine/Casein/Kinesin-Coated Glass

Goal: The goal was to determine whether patterns of microtubules formed when were flown over glass that is coated with poly-l-lysine, casein, and kinesin motor solution.

Materials:

Name	[Stock]	Abbreviation
BRB80		
<i>MgCl₂</i>	100mM	Mg
GTP	25mM	GTP
DMSO	5%	DMSO
Rhodamine Tubulin	20 μ g	
Taxol	1mM	TX
Casein	20mg/mL	CS
D-Glucose	2M	DG
Glucose Oxidase	2mg/mL	GO
ATP	100mM	ATP
Catalase	0.8mg/mL	Cat
Dithiotheritol	1M	DTT
Kinesin (take out of freezer only before needed)		
Poly-l-lysine		PL

Procedure:

1. Prepare **microtubule growth buffer** solution in a microcentrifuge tube:

Volume	Reagent	[Stock]	[Final]
21.8 μ L	BRB80		
1 μ L	Mg	100mM	4mM
1 μ L	GTP	4mg/mL	0.16mg/mL
1.2 μ L	DMSO	5%	0.0024%
25μL	Final Volume		

2. Add 6.25 μ L of growth solution to the aliquot of tubulin (prepare microtubules in the original aliquot of tubulin). **Wrap in foil, expose this to light as little as possible.** This results in a final tubulin concentration of [*Tubulin*] = **3.2mg/mL**. Incubate this on ice for 5 minutes and then incubate at 37°C for 30 minutes. (If incubating in water bath, wrap in parafilm.)

3. Prepare a flow chamber – Use two strips of double sided tape on a 25x75x1mm

coverslip (on the long edges), and stick a 22x22 coverslip on top. Arrange tape so that the flow chamber width is approximately 7mm. (Results in ~100µm height, volume of ~15µL.)

4. Prepare stock solution of poly-L-lysine

Poly-L-lysine 10mg/mL 100µL

100µL of BRB80

1mg of poly-L-lysine

5. Prepare standard solutions: (You can prepare these during MT polymerization). If needed, make diluted stock ATP solution first for lower speed experiments

BRB80CS

Volume	Reagent	[Stock]	[Final]
292.5µL	BRB80		
7.5µL	Casein	20mg/mL	0.5mg/mL
300µL	Final volume		

BRB80PL

Volume	Reagent	[Stock]	[Final]
90µL	BRB80		
10µL	Poly-L-lysine	10mg/mL	1mg/mL
100µL	Final volume		

BRB80CT

Volume	Reagent	[Stock]	[Final]
196µL	BRB80		
2µL	Taxol	1mM	10µM
2µL	Casein	20mg/mL	0.2mg/mL
200µL	Final volume		

Motility Solution (Keep in dark/wrap in foil):

Volume	Reagent	[Stock]	[Final]
75µL	BRB80CT		
1µL	DG	2M	200mM
1µL	GO	20mg/mL	0.2mg/mL
1µL	ATP	100mM	1mM
1µL	Cat	0.8mg/mL	0.08mg/mL
1µL	DTT	1M	100mM
20µL	MT100		
100µL	Final volume		

Antifade solution:

Volume	Reagent	[Stock]	[Final]
95 μ L	BRB80CT		
1 μ L	DG	2M	200mM
1 μ L	GO	20mg/mL	0.2mg/mL
1 μ L	ATP	100mM	1mM
1 μ L	Cat	0.8mg/mL	0.08mg/mL
1 μ L	DTT	1M	100mM
100μL	Final volume		

Note: AF is good for approximately 2 hours from moment of preparation – better to make it again in the middle of the experiment than to make a large batch first.

MT Flow Solution (Keep in dark/wrap in foil):

Volume	Reagent	[Stock]	[Final]
80 μ L	BRB80		
20 μ L	MT100		
100μL	Final volume		

7. Flow in 20 μ L of BRB80PL, wait 15 minutes.
8. Flow in 20 μ L of BRB80CS, wait 5 minutes.
9. Flow in 20 μ L of kinesin motor solution, wait 5 minutes.
10. Flow in 20 μ L of motility solution, wait 5 minutes. **From now on, keep flow cell covered.**
11. Flow in 20 μ L of AF solution twice to wash excess tubulin.
12. Image flow cell immediately.

Results:

Aggregate structures of microtubules were observed.

