WORKSHEET
Actomyosin in vitro motility assay

(Ref.: Kron, S.J., et al. *Methods Enzymol.* **196**, 399-416, 1991.)

Experiment description:

Experiment Number:

I. Solutions:

1. AB buffer	**Volumes:**	**100 ml**	**250 ml**	**1000 ml**
25 mM imidazole-HCl (pH 7.4)		0.17 g	0.425 g	1.7 g
25 mM KCl		0.186 g	0.466 g	1.864 g
4 mM $MgCl_2$ (1 M stock)		0.4 ml	1 ml	4 ml
1 mM EGTA (0.5 M stock)		0.2 ml	0.5 ml	2 ml
1 mM DTT (add fresh from 1 M stock)		100 μl	250 μl	0.154 g

2. 0.2 M KCl/AB	**Volumes:**	**50 ml**	**100 ml**
0.2 M KCl		0.7456g	1.4914g
—add AB buffer—			

3. 0.6 M KCl/AB	**Volumes:**	**50 ml**	**100 ml**
0.6 M KCl		2.237g	4.474g
—add AB buffer—			

4. AB/BSA	**Volumes:**	**50 ml**	**100 ml**
0.5 mg/ml BSA		0.025 g	0.05 g
—add AB buffer—			

5. 0.2 M KCl AB/BSA	**Volumes:**	**50 ml**	**100 ml**
0.5 mg/ml BSA		0.025 g	0.05 g
—add 0.2 M KCl AB buffer—			

6. Blocking solution	**Volumes:**	**2 ml**	**5 ml**
5% BSA	0.5 ml stock*	1.25 ml stock*	
1% Gelatin		100 μl stock**	250 μl stock**
0.2% (v/v) Tween-20		40 μl stock***	100 μl stock***
—add 0.2 M KCl AB buffer—		1.36 ml	3.4 ml

*BSA stock is 20% (w/v) in 0.2 M KCl AB buffer with 0.1% NaN_3.

**Gelatin stock is 20% (w/v) in 0.2 M KCl AB buffer with 0.1% NaN_3.

***Tween-20 stock is 10% (v/v) in water.

7. GOC	**Volumes:**	**2 ml**	**5 ml**	**10 ml**
glucose stock*		40 μl	100 μl	200 μl
catalase**		4 μl	10 μl	20 μl
glucose oxidase***		16 μl	40 μl	80 μl
AB/BSA		1.925 ml	4.815 ml	9.63 ml
β-mercaptoethanol		15 μl	37.5 μl	75 μl

*glucose stock is 30% (1.665M) in water stored on ice. Final concentration of glucose is 3 mg/ml.
**catalase is prepared fresh, stock concentration 20 mg/ml in AB buffer.
***glucose oxidase is prepared fresh, stock concentration 25 mg/ml in AB buffer.

8. ATP	**Volumes:**	**1 ml**	**3 ml**	**Actual prep:**
ATP: always calculate from stock cc.*,e.g.,		3.3 μl	10 μl	
GOC		0.9967 ml	2.99 ml	

*ATP stock is 300 mM aqueous solution of ATP, pH 6.8, in 50 μl aliquots, quic-frozen in liquid nitrogen, and stored at -20˚C or, preferably, at -80 ˚C. Final concentration is adjusted as desired. The numbers are given for 1 mM final ATP concentration.

9. Actin	**Volume:**	**1 ml**	**5 ml**	**Actual prep:**
TRITC-Ph-F-actin stock		2 μl	10 μl ml	
AB/BSA0.998 ml		4.99 ml		

*Note: dilute actin so that a approximate final dilution of 50,000 of the stored F-actin is obtained. Adjust to a concentration that produces an optimal distribution under the fluorescence microscope.

10. Myosin	**Volume:**	**1 ml**	**5 ml**	**Actual prep:**
Myosin stock		3 μl	15 μl	
0.6 M KCl-AB		0.997 ml	4.985 ml	

*Note: the final concentration of myosin should be about 40 μg/ml

11. HMM	**Volume:**	**1 ml**	**5 ml**	**Actual prep:**
HMM stock		0.1 ml	0.5 ml	
AB		0.9 ml	4.5 ml	

*Note: the final concentration of HMM should be about 30-70 μg/ml

12. S-1	**Volume:**	**1 m l**	**5 ml**	**Actual prep:**
S-1 stock		0.05 ml	0.25 ml	
AB		0.95 ml	4.75 ml	

*Note: the final concentration of S-1 should be about 50 μg/ml

13. Additional components of the *in vitro* assay:

a.

b.

c.

Steps: Check:

1. Prepare flow cells (spin-coat or dip-coat 22x40 mm coverslips with nitrocellulose, and allow to dry overnight. Place two Silastic/Parafilm/glass spacers on the NC surface, and a 18 mm^2 coverslip on top) _____
2. Pipet aliquots of solutions (150 μl) in Eppendorf centrifuge tubes _____
3. Degas solutions in desiccator _____
4. Place the flow cell in a 30-ml beaker, and start adding the solutions: _____
   (Common sequence:
   a. 50 μl myosin (or HMM or S-1) applied from both ends of the flow cell, wait 1 min.
   b. 100 μl AB/BSA
   c. 100 μl actin, wait 1 min.
   d. 100 μl AB/BSA
   e. 100 μl GOC)
6. Oil the objective, and place the flow cell on the microscope stage _____
7. Turn on the camera and bring actin filaments in focus. Start recording. _____
8. Add 100 μl ATP with a micropipet while drawing fluid with filter paper at the other end. _____

Tepmperature:

Video Recording: **Tape Number:**

Experiment	Step	Tape Time
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Notes/Remarks/Modifications: