WORKSHEET
Fluorescent labelling of F-actin

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

Number:

Number of Actin preparation:

A. 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. Rhodamine-phalloidin solution
Stock from Molecular Probes, Eugene, OR
Stock concentration: 3.3 μM
Solvent: methanol

3. Alternative rhodamine-phalloidin stock
From Sigma Chemical Company, St. Louis, MO
Product Number: P1951 ("Phalloidin-TRITC Lebeled; Mixed isomers"; 100 μg)
FW: 1305.6
Stock concentration: 300 μM
Solvent: Ethanol (absolute)
Preparation: -Add 255 μl* absolute ethanol to a vial containing TRITC-Ph
-Vortex and sonicate extensively
-Store at -20 °C

B. Steps:

1. Dry 94 μl of TRITC-Ph (Molecular Probes) in Eppendorf tube.
2. Dissolve pellet in 2 μl Ethanol.
3. Add 290 μl AB bufer, and vortex extensively (approx. 30 sec)
4. Add 10 μl of 1 mg.ml F-actin in AB, mix well. Store on ice for weeks.

Alternatively:

1. Add 1 μl of TRITC-Ph stock (Sigma) to 296.5 μl AB. Vortex extensively. (Final concentration of TRITC-Ph is 1 μM)
2. Add 2.5 μl F-actin (4 mg.ml; usual concentration after actin preparation). (Final actin concentration: cca 0.8 μM)
3. Mix well. Store on ice for weeks.

Alternatively:

1. Carry out labelling on fresh, unpolymerized G-actin in polymerization buffer.

C. Microscopic testing of fluorescent actin filaments

1. Add 2 μl of TRITC-Ph-F-actin stock to 1 ml of AB containing 100 mM DTT or b-mercaptoethanol. Place 5 μl of this mixture on coverslip, and examine under epifluorescence microscope.

D. Notes/Modifications:

*General equation for calculating concentrations:

Weight of chemical = Molecular weight * Desired concentration * Volume
(to be measured out (MW or FW) (in Moles/liter) (liters)
in grams)