## In vivo Actin Polymerization Assay in Developed Dictyostelium cells

## Solutions:

- 2X Actin Buffer (40 mM KPO<sub>4</sub>, 20 mM PIPES, 10 mM EGTA, 4 mM MgCl<sub>2</sub>, pH 6.8)
- DB Buffer (5 mM Na<sub>2</sub>HPO<sub>4</sub>, 5 mM KH<sub>2</sub>PO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 200 μM CaCl<sub>2</sub>, pH 6.5)
- □ PM Buffer (10 mM Phosphate Buffer, 2 mM MgSO<sub>4</sub>). Prepare from a stock of 10X Phosphate buffer, pH 6.3)
- □ Assay Buffer

1X Actin Buffer	<i>C</i> /2 =	mL 2X Actin Buffer
3.7 % Formaldehyde	<i>C</i> /10 =	mL 37% Formaldehyde
0.1% Triton X-100	<i>C</i> /100 =	mL 10% Triton X-100
1X Rhodamine-Phalloidin	<i>C</i> /320 =	μL 80 μM Rh-Ph stock
Raise to final volume $C$ mL with ddH <sub>2</sub> O.		

## **Development of the Cells**

□ Count number of cells. Spin cells at 2000 rpm for 5 mins. Wash cells 2X with

DB and resuspend them to  $2x10^7$  cells/mL in 5 mL.

Volume of cells required for starvation in mL = Total # cells required / # cells.mL

- $\Box$  Shake cells at 100 rpm for 1 hour.
- $\square$  Pulse cells with 50 nM cAMP (100 µL) every 6 mins for 4 hrs. Total volume to

be added to the cells is 8 mL. So 10 mL cAMP is required per flask.

 $\square$  Basolate cells with 3 mM caffeine in PM Buffer for 15-30 mins (1X10<sup>7</sup> cells/mL)

and increase speed to 200 rpm.

- □ Aliquot 1 mL Assay Buffer into eppendorf tubes.
- □ Spin cells at 2000 rpm 3 mins. Resuspend cells in 3 mM caffeine in PM Buffer to 3X10<sup>7</sup> cells/mL and shake at 200 rpm for 10 mins. Vol of culture \_\_\_\_mL\*2x10<sup>7</sup> cells/mL = 3x10<sup>7</sup> cells/mL\*X X=\_\_\_\_mL

## Assay

- $\Box$  Take 2 mL cells, shake at 200 rpm, at time 0, add 20  $\mu$ L 100  $\mu$ M cAMP.
- At time 0, 4, 8, 15, 30, 45, 60, 75, 90, 105, 120, 180 seconds take 100 μL aliquots and add to 1mL assay buffer.
- $\Box$  Cover with aluminum foil and shake to fix at room temperature for 1 hr.
- □ Spin 14K for 10 min, keep pellet.
- □ Add 1 mL MeOH, wrap in aluminum foil and shake overnight.
- □ Read samples on Fluoro-Max. Excitation 540 nm, emission 570 nm, slitwidth 3mm.

Catalog # of Chemicals from SigmaRhodamine-PhalloidinP-1951CaffeineC-0750cAMP sodium saltA-6885 (1 g)