STAINING F-ACTIN WITH PHALLOIDIN

Materials and Reagents:

S mix

	[stock]	Volume added (μL)	[final]
Na-Phosphate buffer pH 7.5	0.8M	250	0.2mM
MgCl2	1M	1	1mM
SDS	1%	4	0.004%
dH20	-	745	-

Na-phosphate: 8.1 ml 1M Na2HPO4, 1.9 ml 1M NaH2PO4, 2.5 ml dH20
PBBT: PBS, 0.5% BSA, 0.5% Tween-20
Mounting solution: 90% glycerol, 10% PBS, 1 mg/ml phenlyenediamine
Rhodamine-phalloidin (Molecular Probes)

Procedure:

1) Wash worms off the plate in water, PBS, or M9.
2) Wash 2 times to remove excess bacteria (spin down at 3000rpm X 1min).
3) Remove as much supernatant as possible.
4) Add 10μL worms to an eppendorf tube.
5) Freeze tube in liquid nitrogen.
6) Lyophilize worms by spinning in speed vac (~5 minutes).
7) Add 3-4 drops ice cold acetone and put in freezer 3 min.
8) Remove acetone with pipetman (or aspirate carefully) and speedvac to remove residual acetone.
9) Add 2U rhodamine-phalloidin to an eppendorf tube and speedvac to evaporate methanol. Resuspend in 20μL S mix.
10) Resuspend dry worms in S mix/phalloidin.
11) Stain in the dark at RT for at least 30 min.
12) Wash 2X in 1 mL PBBT.
13) Resuspend in ~20 μL PBS.
14) For viewing, mix equal volumes of worms and mounting medium.
15) Mount on an agar pad and examine under the microscope.

Tips/Troubleshooting:

1) Worms often remain stuck together after lyophilizing. Mix by pipeting up and down with a pipetman, but be sure to cut the tip to give a wider opening so that worms will not be sheared
2) Mounting medium is not strictly necessary unless you plan to store the slides for some time (days). Worms can be mounted in PBS on an agar pad.
3) Molecular Probes sells phalloidin conjugated to different fluorophores depending on what you need to co-stain with

References

1) This protocol is adapted from Michael Koelle's (http://cobweb.dartmouth.edu/~ambros/worms/index.html)

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