Phalloidin staining for epidermis:

Never put your embryos in MeOH - phalloidin staining will not work afterwards.

1) Wash embryos 3 x 10min. in TBST on rotator at room temp.

2) Evaporate out MeOH from Phalloidin on 37 degree hotblock
(MeOH will cause Phalloidin staining not to work in Xenopus so make sure ALL MeOH evaporates off, cover tube in foil since phall. is light sensitive)

3) Resuspend dry Phalloidin in TBST [10ul Phalloidin/500ul TBST]

4) Incubate embryos in 500ul Phalloidin/vial on nutator at room temp. for 1 - 2 hours (for epidermis).

5) Wash embryos 3 x 10min. in TBST on rotator at room temp.

Notes for Jenn-

We use Alexa Flour 488 Phalloidin (cat. # A12379 from Invitrogen) or Alexa Flour 555 Phalloidin (cat. #A34055/Invitrogen)

TBST:
Tris Cl ph7.5 10mM
NaCl 155mM
Triton X 1%

The data sheets that come with the Phalloidins that we use don't say that you have to evap. the MeOH out of them before diluting. I've always done it, but another guy in my lab never does it and our phalloidin staining looks the same. It will definitely NOT work if you dehydrate your embryos in MeOH though.

2nd protocol (Aliva's Protocol)

Fix Embryos for 10' in the following:

	For 5ml
3.7 % Formaldehyde	500ml 37%
0.25% Glutaraldehyde	25ml 50%
0.1% Tween-20	25ml 20%
in PBS	250ml 20X
Water to 5ml	

Follow with 3 x 10' washes in PTW (0.1%)

Stain with Rhodamine-Phalloidin in PTW (10ml /500ml PTW)

After staining dehydrate into Isopropanol then clear in BB:BA (2:1)