PROTOCOL FOR PHOTOOXIDATION WITH PHALLOIDIN-EOSIN

Fix cells 5 minutes @ 37°C then 15 minutes on ice in:

A) 4% paraformaldehyde in CSB*

B) 4% paraformaldehyde with 0.2% gluteraldehyde in CSB*

*CSB:

137 mM NaCL

5 mM KCl

1.1 mM Na_2HPO_4

0.4 mM KH_2PO_4

4 mM $NaHCO_3$

2 mM $MgCl_2$

5.5 mM glucose

2 mM EGTA

5 mM Pipes

Wash with CSB 4 x 4 minutes and then glue coverslip to dishes.
Add working buffer for 5 minutes:

0.5% cold water fish gelatin
50 mM glycine
0.05 % triton
5mM potassium cyanide
in CSB

Stain with phalloidin in working buffer for >1 hour:

A) eosin-phalloidin diluted 20x
B) eosin-phalloidin diluted 20x with rhodamine-phallodin diluted 100x

Wash with CSB 4 x 4 minutes and observe with confocal

Fix with 2% gluteraldehyde for 5 minutes in CSB on ice

Wash with 0.1M sodium cacodylate 4 x 2 minutes.

Add 0.5mg/ml DAB in 0.1M sodium cacodylate and photooxidize

Wash with 0.1M sodium cacodylate 4 x 2 minutes.

Post-fix with 1% OsO_4 in 0.1M sodium cacodylate for 30 minutes.

Rinse in DDW

Dehydrate in ETOH series.

Infiltrate with 50:50 Durcupan-ETOH for 30 minutes

Infiltrate with 100% resin 1 hour x2.

Polymerize at 60°C for 24 hours.

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what do you think? Is it a good idea to do everything in CSB, especially the DAB-reaction, and the osmication with all the glucose and EGTA and stuff around?

Did you find the negatives? They were from an experiment that was done on 2/12/98, the numbers are around 78600-78700