Fixation procedure (MeOH/acetone)

1. spin down cells 4 krpm 4°C, 2min

2. resuspend 1 ml ice cold water

3. transfer to 1.5 ml tube

4. spin down 4 krpm, 1 min

5. take off water, vortex, add 1 ml methanol (from freezer)

6. leave on ice for >10 min

7. spin down 4 krpm, 1 min

8. pour off methanol, vortex

9. add 1ml acetone (from freezer) - store at -20°C (ok at least for a few days)

10. To mount cells, spin down and leave small amount of acetone so that cells are quite concentrated.

11. Spread cells on slide (10ul) with yellow tip (try to spread thinly) - cells will dry instantly.

12. Add 10 ul mountant and coverslip (press moderately hard on coverslip). If using agarose mountant allow to set (can put in fridge).

13. Keep slide in dark until ready to view

Comments:

mount in normal way (ie concentrate cells by eg spinning down 0.2 ml, leave 20 ul or so for resuspension, spread on slide, mount in agarose).

Mountant:

Add 0.06 g LMT agarose to 10 ml 0.1M Tris-acetate pH 8.5 and melt in microwave (use 1.2% if aqueous cells are to be used)

Add DAPI to 50-125 ng/ml
Store in dark (4°C?)

Before use melt (at 65°C) then cool to 37°C.

Agarose mountant (which seems to preserve YFP fluorescence of Pol1-YFP)

25/5/05 v2

1 g DABCO (antifade*)
10 ml 0.1 M Tris-acetate pH 8.5

Dissolve, re pH to 8.5 with acetic acid
Make up to 100 ml

Add 1.0 g LMT agarose and melt in microwave (use 1.2% if aqueous cells are to be used)

Add DAPI to 50-125 ng/ml
Store in dark (4°C?)

To use

1. Melt agarose and cool to 37°C
2. Spread cells in acetone in normal way (use ordinary slide – no need to use polylysine coated)
3. Add 10 ul agarose moutant
4. Drop on coverslip - push firmly to get a monolayer of cells – leave 5 min before viewing to allow time to set.
5. May help to seal (or put a few dabs of) with nail varnish on edge of cover slip to stop drag of objective on coverslip moving the cells about (slide drying out isn't problem).

* doesn't actually seem to make a lot of difference to the rate of fading of GFP etc but does help when used with conventional fluor 2ndary antibodies.

For visualization of septa, add methyl blue to 20 ug/ml (this may interfere with seeing faint FP fluorescence)