Immunofluorescence labeling

Solutions:

MTSB (1x)
50 mM PIPES 15,1 g (PIPES free acid, mw=302,4)
5 mM $MgSO_4.7H_2O$ 1,23 g
5 mM EGTA 10 ml of 0,5 M stock solution
add solid KOH to dissolve PIPES first
final volume 1000 ml
pH=6,9 (use KOH to set)

EGTA (0,5 M stock solution)
3,8 g EGTA in 10 ml H_2O, add solid KOH until clear
make to 20 ml, pH=8

PBS
0,14 M NaCl 8,0 g
2,7 mM KCl 0,2 g
6,5 mM $Na_2HPO_4.2H_2O$ 1,15 g
1,5 mM KH_2PO_4 0,2 g
make with dH_2O up to 1000 ml, pH=7,3

Formaldehyde fixation
18 ml MTSB
2 ml 37% formaldehyde

Wax (PEG + 1-hexadecanol = 9+1 w/w)
melt PEG 400 distearate at 65°C, add 1-hexadecanol
stirr 3-4 hrs
pour wax into Aluminium containers, cool at RT

First antibody
Dilute in PBS, add 10 µl of BSA stock solution per 1 ml of PBS

Second antibody
Dilute in PBS, add 10 µl of BSA stock solution per 1 ml of PBS

BSA stock
100 mg in 1 ml dH_2O, store 100 µl aliquots at –20°C

DAPI stock (100 mM)
1 mg in 10 ml H_2O

DAPI working solution
2 µl of 100 mM stock solution in 2 ml PBS

Mounting medium
100 mg p-phenylenediamine in 10 ml PBS, add 90 ml of glycerol
set pH to 8,0 with carbonate/bicarbonate or Tris pH=8,0
store at -20°C

Procedure:

1.	Formaldehyde fixation	60 min
2.	MTSB	30 min
3.	PBS	15 min
4.	Dehydratation	
	30 % ethanol in PBS	30 min
	50 % ethanol in PBS	30 min
	70 % ethanol in PBS	30 min
	90 % ethanol in PBS	30 min
	97 % ethanol	30 min
	Toluidine blue in ethanol	10 min
5.	Embedding	
	97 % ethanol	10 min
	wax + ethanol (1:1)	overnight
	100 % wax	1-2 hrs
6.	Sectioning	
	sections attached to glycerol-albumine coated slides	overnight
7.	Dewaxing and Rehydratation	
	97 % ethanol	3x10 min
	90 % ethanol in PBS	10 min
	50 % ethanol in PBS	10 min
	PBS	2x10 min
8.	Labeling	
	First antibody (at RT)	1 hr
	PBS	
	Second antibody (at RT)	1 hr
	PBS	10 min
	DAPI	10 min
	PBS	10 min
	Toluidine Blue (0,01 % in PBS)	10 min
	PBS	10 min
9.	Apply mounting medium and coverslide, seal with nail varnish	