AP STAINING OF CELLS

1) Wash cells with PBS (- Ca^{++}, Mg^{++})

2) Fix with cold Glutaraldehyde (0.5%)

- 5-10' R.T.

remove fixative

If double-staining begin at this stage at end of X-gal staining

3) Wash cells with PBS (- Ca^{++}, Mg^{++})
- 3 x

4) Incubate with AP buffer (0.1 M Tris pH 9.5) + Levamisole (1 µl/1.5 ml)

65°C for ~20'

-can use oven (longer time) or water bath

5) Cool to RT

- Levamisole 1 µl in 1.5 ml AP buffer/well of 6 well

Incubate 10' RT

Remove

6) Incubate with AP stain (0.6 ml/well of 6-well) (in dark) at RT until colour change (~20 - 60')

AP Stain (BCIP / NBT (Vector Kit IV))

BCIP	2 drops
NBT	1 drop
Levamisol	1 drops
$MgCl_2$	2 drops
Tris pH 9.5 (0.1 M)	to 5 ml

7) Remove AP stain

6) Wash cells with H_20