

Date: _____

Enhancer Trap Staining with X-gal (Adapted from W.H. Biggs 8/89 protocol)

This is carried out in dissecting plates with 10-20 μ l of each solution per well.

1. Dissect eye/antennal disc - optic lobe complex from larvae in 1X PBS.

Samples:

2. Fix complex in 0.8% glutaraldehyde/100mM cacodylate pH 7.3 for 15 - 25'.
(30 μ l 25% glutaraldehyde + 970 μ l cacodylate)
3. Wash complex with PBS for at least 5' (can do for longer while waiting for other samples).
4. Stain complex in 0.2% X-gal/FeNaP pH 7.2 overnight at RT.
(25 μ l 8% X-gal (in DMSO) + 975 μ l FeNaP)
Wrap staining plate in parafilm to prevent evaporation.

Start time: _____

} Total time overnight: _____

End time : _____

5. Wash complex in PBS for at least 1h (can leave all day if necessary).
6. Mount in 100% glycerol and seal with nail-polish.

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Solutions:

Staining Buffer:

10 mM NaP pH 7.2	1 L	100 ml
150 mM NaCl	8.77 g	0.87 g
1 mM MgCl ₂ ·6H ₂ O	0.2 g	0.02 g
3.1 mM K ₄ [Fe ^{II} (CN) ₆]	1.37 g	0.137 g
3.1mM K ₃ [Fe ^{III} (CN) ₆]	1.02 g	0.102 g
0.3% Triton	3 ml	300 μ l

* Meaning if you want a copy of this, you must buy Gail a taco.