X-GAL STAINING [Frozen Cryostat Sections]

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DAY 1

- Mount tissue in OCT. We use Martin Heisenberg's fly collars to obtain oriented sections of fly heads, but it is possible to mount adult tissue without the benefit of these devices. Freeze samples in dry ice and mount on cryostat chucks. Collect 14 um cryostat sections on Fisher SuperFrost Plus slides. Do not let sections dry longer than it takes to collect them.
- Fix immediately after sectioning for 7 minutes in 4% Paraformaldehyde/1X FIX BUFFER.
- Wash 3 times 30 minutes, X-GAL BUFFER. If you are doing a lot of sectioning, fix and transfer to X-GAL BUFFER as you go along, then do 3 times 30 minute washes in X-GAL BUFFER.
- About ten minutes before the washes are done, prewarm to 37 degrees C sufficient X-GAL WASH BUFFER for the amount of STAINING SOLUTION you will be preparing (0.5 ml to 1 ml per slide). Add other components of staining solution and hold at 37 degrees until ready to incubate slides
- Transfer slides to humidified, foil-covered chamber and apply 0.5 ml to 1 ml STAINING SOLUTION per slide. Incubate ten minutes to overnight at 37 degrees (typically) or 25 degrees.

DAY 2

- Prewarm 100 ml X-GAL WASH BUFFER to 37 degrees.
- Return slides to staining jar and wash in X-GAL BUFFER 2 times 5 minutes at 37 degrees (to discourage X-GAL crystal formation).
- Rinse sections 2 minutes in 1XPBS.
- Rinse quickly with ultrapure water.
- Remove slides from staining jar, and air dry 5 minutes.
- Mount sections by applying 150 ul DAKO Glycerolgel preheated to 55 degrees C and applying a 24 x 60 mm glass coverslip.
- View sections using Nomarski/DIC optics.

SOLUTIONS

1. FIXATIVE

4% Paraformaldehyde
1X X-Gal Fix Buffer

Mix equal parts 2X FIX BUFFER and 8% PARAFORMALDEHYDE. Check final pH with a piece of pH paper to ensure that pH is approximately 7.0-8.0.

2. 2X X-GAL FIX BUFFER	100 mL
200 mM Sodium Phosphate pH7.4	32.4 ml 0.5M dibasic Sodium Phosphate 7.6 ml 0.5M monobasic Sodium Phosphate
10 mM EGTA	2 mL 0.5 M EGTA
4 mM MgCl2	0.4 mL 1 M MgCl2

3. 8% PARAFORMALDEHYDE

Autoclave 450 mL ultrapure water in a 500 ml bottle. Transfer bottle to a fume hood, allow to cool for a few minutes, and add 40 g paraformaldehyde and 50 uL 10 N NaOH, and a clean stir bar. Stir until dissolved on a magnetic stir plate, bring volume to 500 mL with ultrapure water and sterile filter. Good for 1 week if kept at 4 degrees.

4. X-GAL WASH BUFFER	1000 ml
0.1 M Sodium Phosphate pH 7.4	162 ml 0.5M dibasic Sodium Phosphate 38 ml 0.5M monobasic Sodium Phosphate
2 mM MgCl2	2 ml 1 M MgCl2
0.01% Sodium Deoxycholate	0.1 g
0.02% Nonidet P-40	200 ul

5. STAINING SOLUTION	Stocks	For 5 mL	For 50 mL
1 mg/ml X-GAL	40 mg/ml	125 uL	1.25 mL
1 mM Spermidine HCl	100 mM	50 uL	500 uL
5 mM K3Fe(CN)6	500 mM	50 uL	500 uL
5 mM K4Fe(CN)6-6H2O	500 mM	50 uL	500 uL

X-GAL WASH BUFFER	1X	to 5 mL	to 50 mL