

Whole Mount X-gal Staining of Mouse Embryos

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Solutions:

2X Wash buffer (500 ml)

<u>Stock Solution</u>	<u>mL</u>	<u>Final Concentration</u>
1M MgCl ₂	2	4 mM
1% sodium deoxycholate	10	0.02%
10% NP-40	2	0.04%
1M PBS pH 7.3	100	0.2 M
dH ₂ O	To 500 ml	

X-gal Staining buffer (10 ml)

<u>Stock Solution</u>	<u>uL</u>	<u>Final Concentration</u>
50 mg/ml X-gal in DMF	200	1 mg
200 mM potassium ferrocyanide	250	5 mM
200 mM potassium ferricyanide	250	5 mM
1M Tris pH 7.4	200	20 mM
1X Wash buffer	To 10 ml	

X-gal fixative (20 ml)

<u>Stock Solution</u>	<u>mL</u>	<u>Final Concentration</u>
4% PFA	5	1 %
1M MgCl ₂	0.04	2 mM
0.5M EDTA (pH 8.0)	0.2	5 mM
10% NP-40	0.04	0.02%
dH ₂ O	To 20 ml	

Notes:

- a) PBS = phosphate buffered saline
- b) DMF = dimethylformamide
- c) PFA = paraformaldehyde
- d) sodium deoxycholate is from Sigma (Cat# D6750)
- e) X-gal (10g) is from Gold BioTechnology (Cat #X4281C10; \$199)
- f) 10% NP-40 and 1% sodium deoxycholate = store at 4°C; make fresh monthly
- g) To make 4% PFA:
 - a. dissolve 4 g paraformaldehyde in 100 ml warm PBS
 - b. cool and adjust to pH 7.0
- h) To make 200 mM potassium ferrocyanide (Sigma Cat# P3289; MW = 422.4 g/mol)
 - a. Add 0.4224 g to 5 ml dH₂O
 - b. Store in dark at 4 °C (good for ~1 month)
- i) To make 200 mM potassium ferricyanide (Sigma Cat# P3667; MW = 329.2 g/mol)
 - a. Add 0.3292 g to 5 ml dH₂O
 - b. Store in dark at 4 °C (good for ~1 month)

Procedure:

- 1) Wash embryos in PBS 2X at room T to remove any media or serum
 - a. Larger embryos (> E13.5) may need to be dissected to allow penetration of staining solution
- 2) Prefix embryo in X-gal fixative for 20-90 min on ice
 - a. For E13.5-14.5, fix for 20 min
 - b. For E15.5+, fix for 90 min
- 3) Wash 2X 20 min with 1X Wash buffer at room T
- 4) Add X-gal staining buffer (enough to cover embryo)
 - a. Cover in foil and nutate in dark at 37 °C (~2-18 hours)
- 5) Rinse embryo 2X 30 min with 1X Wash buffer at room T
- 6) Cut tails for genotyping
- 7) In 12-well plate, one embryo per well, postfix embryo in 4% PFA overnight or longer at 4 °C
 - a. For E13.5-14.5, fix for 2 days
 - b. For E15.5+, fix for 3 days
- 8) Wash 2X in PBS
- 9) Embryos can be analyzed as whole mounts or processed and embedded as for normal histology