

β -galactosidase Staining of Mouse Embryos

(Rivera Lab)

(Adapted from R. Behringer's Protocol)

Fix: E6.5–E7.5, 5'; E8.5–9.5, 10'; E10.5–12.5, 15' and E13.5–E14.5, 20'.
(0.2% Glutaraldehyde, 2% Formalin, 5mM EGTA and 2mM MgCl₂ in 0.1 M Phosphate buffer pH 7.3)

Reagent	1.0 ml	5.0 ml	10.0 ml	20.0 ml
MiliQ H ₂ O	0.69 ml	3.43 ml	6.86 ml	13.72 ml
0.5 M Phosphate pH 7.3	0.20 ml	1.00 ml	2.00 ml	4.00 ml
0.1 M EGTA pH 8.0	50.0 μ l	0.25 ml	0.5 ml	1.0 ml
37% Formaldehyde	54.0 μ l	0.27 ml	0.54 ml	1.08 ml
25% Glutaraldehyde	8.0 μ l	40.0 μ l	80.0 μ l	160.0 μ l
1 M MgCl ₂	2.0 μ l	10.0 μ l	20.0 μ l	40 μ l

Rinse: 3 times, about 30 minutes each.

(0.1% Na-deoxycholate, 0.2% IGEPAL, 2mM MgCl₂ in 0.1 M Phosphate buffer pH 7.3)

Reagent	5.0 ml	10.0 ml	20.0 ml	40.0 ml
H ₂ O	4.0 ml	8.0 ml	16.0 ml	32.0 ml
0.5 M Phosphate pH 7.3	1.0 ml	2.0 ml	4.0 ml	8.0 ml
1 M MgCl ₂	10.0 μ l	20.0 μ l	40.0 μ l	80.0 μ l
IGEPAL	10.0 μ l	20.0 μ l	40.0 μ l	80.0 μ l
Na-Deoxycholate	0.005 g	0.010 g	0.020 g	0.040 g

Stain: Room temperature or 37 °C, usually overnight.

(1 mg/ml X-gal, 5 mM Potassium ferricyanide, 5 mM Potassium ferrocyanide in rinse)

Reagent	5.0 ml	10.0 ml	20.0 ml	50.0 ml
25 mg/ml X-gal in DMSO	0.2 ml	0.4 ml	0.8 ml	2.0 ml
K Ferricyanide (red)	0.00825g	0.0165g	0.0330g	0.0825g
K Ferrocyanide (yellow)	0.0092g	0.0184g	0.0368g	0.0376g

After staining, rinse in PBS to remove yellow coloration. Photograph. Store in 70% ethanol in refrigerator.

Note: Background may be a problem in mouse fetuses older than E12.5. Also, internal tissues may not stain in large embryos.