

LacZ staining protocol (May 2000)

Lac Z staining of postimplantation embryos, tissues and cells (Nagy lab).

1. 0.1 M phosphate buffer pH 7.3

115 ml of 0.1 M Sodium Phosphate monobasic (MW 155.99) (1.8 g per 500 ml)
385 ml of 0.1 M Sodium Phosphate dibasic (MW 268.07) (10.32 g per 500 ml)

This mixture should give a pH of 7.3 Alternatively:

141 g Na₂HPO₄ + 8 ml 85% phosphoric acid per 2 L (pH 7.3 to 8.5) – try pH 8.0

A background staining is seen in yolk sac of day 10 embryos and a thin stripe of staining is observed in hindbrain of day 12 embryos. Increasing pH of Phosphate buffer might help to reduce background. The procedure works at pH 8.5.

2. Lac Z fixative solution

Might be prepared ahead except for the *gluteraldehyde* (added fresh each time)

0.4 ml	25 % gluteraldehyde (Sigma G6257)
2.5 ml	100 mM EGTA (pH 7.3) MW 380.4 (3.804 g per 100 ml)
(1.0 ml	250 mM EGTA (pH 7.3))
0.1 ml	1M MgCl ₂
	0.1 M Phosphate Buffer pH 7.3 or PBS up to

50.0 ml

3. Wash buffer

0.4 ml	1M MgCl ₂
2.0 ml	1% Sodium deoxycholate (NaDC; make up in water, store at 4 ° C)
2.0 ml	2 % Nonidet-P40 (make up in water, store at 4 ° C)
195.6 ml	0.1 M Phosphate Buffer pH 7.3 or PBS

200.0 ml

4. X-gal stain

Final concentration

1-2 mg/ml X- gal
(e.g. from Fermentas # R0401 www.fermentas.com 1-800-340-9026)
2 mM MgCl₂
5 mM K₄Fe(CN)₆ x 3H₂O
5 mM K₃Fe(CN)₆
in wash buffer or PBS without Ca and Mg

Option 1.

2.0 ml 25mg/ml X-gal stock solution in di-methyl-formamide

(stored in the dark, at -70°C or -20°C)
 0.106 g Potassium Ferrocyanide (Sigma P9387)
 0.082 g Potassium Ferricyanide (Sigma P8131)

Wash buffer or PBS (without Ca and Mg) + 2 mM MgCl_2
 (deoxycholate and Nonidet-P40 can be omitted in the wash buffer to increase the
 intensity of staining) up to

50.0 ml

Option 2.

Concentrated (100 or 250 mM) solution of potassium ferrocyanide and ferricyanide
 might be prepared ahead and stored foil-covered at room temperature. All components
 are mixed directly prior to staining.

Components:	Volume	Final
X-gal 20 mg/ml stock	5 ml	500 ul
1M MgCl_2	100 ul	10 ul
100 mM $\text{K}_4\text{Fe}(\text{CN})_6 \times 3\text{H}_2\text{O}$	2.5 ml	250 ul
100 mM $\text{K}_3\text{Fe}(\text{CN})_6$	2.5 ml	250 ul
PBS	to 50 ml	5 ml
		2 mg/ml
		2 mM
		5 mM
		5 mM

After staining, solution may be reused following filtration through Whatman paper and
 stored at -20°C in the dark.

LacZ staining of postimplantation stage embryos

1. Dissect embryos in PBS containing 0.9 mM CaCl_2 and 0.5 mM MgCl_2 in Petri dishes under a dissection microscope.
2. Transfer of embryos is achieved using a 1ml pipetman and a blue tip with the end cut off or Samco Transfer pipettes (Cat. # 222-20S). Embryos are fragile and will be damaged if transferred with forceps.
3. Rinse embryos in 0.1 M Phosphate buffer at room temperature (optional).
4. Fix embryos at room temperature with optional mixing for 30 to 90 minutes depending on the size of the embryo. (For example day 9.5 embryos are fixed for 60 minutes or 30 min on ice with shaking).
5. Wash embryos three times for 15-30 minutes each at room temperature in wash buffer.
6. Stain embryos in the dark with mixing for 2 hours to overnight depending on a level of Lac Z activity. Staining is usually performed at 37°C , but if done at $27-30^{\circ}\text{C}$ background staining would be significantly reduced.
7. Stain solution is replaced with wash buffer and samples are stored at 4°C (staining will intensify in wash buffer with time). Embryos can also be fixed after staining in fresh solution of 4% formaldehyde (Fisher F 79-1) in PBS.

For samples to be cryosectioned after washing, cryoprotect in 15% sucrose in PBS for 1 hour at 4°C , then in 30% sucrose in PBS overnight at 4°C . Incubate in O.C.T. at 4°C for 1 hour or more prior to embedding in O.C.T. over dry ice.

For day 12.5 embryos and older as well as for adult tissues the samples are often sectioned sagittally after 30-60 minutes in pre-fix solution (*prepared fresh*): e.g. 2% Paraformaldehyde (PFA) or 2% formaldehyde added to the regular X-gal 0.2% Gluteraldehyde fixative. Freshly made 4% PFA may be used as well.

1. Keep the embryos in fixative for 30-60 minutes at room temperature (with shaking). Larger organs are fixed for at least 1 hour (or longer). Skin and scalp need to be removed from older fetuses and newborns.
2. Cut the embryo sagittally using a straight razor blade
3. Fix an additional 30 minutes to 1 hour on ice in the same fixative.
4. Replace the fixative with fresh LacZ fix (gluteraldehyde), keep for additional hour (optional).
5. Wash x3 for 15-30 minutes in wash buffer (samples to be cryosectioned could be washed in PBS).
6. Proceed with LacZ stain at 37° C for 30 minutes + or room temperature overnight, protecting from light, best if done with shaking.
7. Cryoprotect samples to be cryosectioned. Preserve stained embryos in 10% Formalin.

Lac Z staining of cells

1. Rinse cells with PBS x2
2. Add fresh fix (160 ul of 25% Gluteraldehyde in 20 ml of PBS). Fix for 5- 10 minutes at room temperature.
3. Wash with PBS x3
4. Add X-gal stain, protect from light, incubate at RT or 37 C.

Genotyping of mice by Lac Z staining of ear punches

Earpunch mice into PBS in a 96-well plate (50 ul per well).
Fix 15 minutes to 1 hour in 0.2% gluteraldehyde in PBS.
(Shortcut: add equal volume of 0.4% gluteraldehyde in PBS)

0.4 % Gluteraldehyde = 2x stock:
80 ul 25% gluteraldehyde
5 ml PBS

Wash 5 minutes (or more) in PBS 1x or 2x. Stain with X-gal stain (may reuse old stain) at 37°C (or at RT), protecting from light. *Staining is usually visible after several hours at 37 C, can be left O/N at room temperature.

> Dear listers,
> even though we do a lot of X-Gal staining in ES-cells and embryos, we have
> relatively little experience with adult tissues. There seems to be quite high
> endogenous activity in kidney, spleen, uterus and testis, but other organs show
> also some 'background staining' which seems to be variable. Does anyone have
> experience with staining in wild type mice? Are there strain differences?
> Searching the list I noticed that a similar question was posted by bradley Bloemker
> in 97, but apparently no one answered.

Date: Wed, 7 Nov 2001 09:09:40 -0800 (PST)

Author: Ryan Blair Rountree <routree@stanford.edu>

Subject: Re: X-Gal staining of adult tissues

Body: I have done some Xgal staining of adult tissues and the best thing I found for reducing background staining was to stain at 4 degrees. This seems to cut endogenous activity a lot but the transgenic lac Z is still quite active. Make sure to stain some controls without lacZ to compare while staining.

Fix each set of organs in 25 mls 4% PFA 1hr 30min at 4 degrees w/shaking

Rinse 2 X's 1 X PBS 20-30 min 4 degrees

Rinse 1 X 30 min in buffer for lacZ staining

Stain 4 degrees overnight - 2 days.

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Date: Thu, 08 Nov 2001 07:56:13 -0500

Author: Thom Saunders <tsaunder@umich.edu>

Subject: Re: X-Gal staining of adult tissues

Body: To reduce background staining, make sure that you increase the pH of staining above 7.5 to inhibit endogenous galactosidases. See:

Young DC, Kingsley SD, Ryan KA, Dutko FJ.

Selective inactivation of eukaryotic beta-galactosidase in assays for

inhibitors of HIV-1 TAT using bacterial beta-galactosidase as a reporter

enzyme. Anal Biochem. 1993 Nov 15;215(1):24-30.

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