

X-gal Staining of Frozen Sections

(does not work on paraffin sections)

1. If sections have been frozen, allow them to thaw.
2. If conserving reagents and willing to take more time: Making sure that the slide is dry, use a “PAP pen” hydrophobic slide marker to encircle the group of sections on the slide; allow to dry to several seconds

If slides can be incubated in a small enough slide holder, forego the PAP pen.

3. Put slides in a glass slide rack and fix in ice cold X-gal fixative solution for 4 minutes in a chemical fume hood.

The fixing itself can be at room temperature.

4. Transfer slide rack to PBS, let sit for 1 minute.
5. Transfer slide rack to fresh PBS, let sit for 10 minutes.
6. Dilute 40 mg/ml X-gal 1:40 into staining solution immediately before use. If using PAP pen, remove slides from slide rack one by one, drain as much PBS as possible, and apply diluted X-gal solution to the area encircled by the PAP pen.

It takes approximately 800 μ l per slide using PAP pen. If not using PAP pen, simply immerse all slides in the X-gal staining solution.

X-gal is not cheap. If using the immersion approach, use a slide rack or holder small enough to minimize empty slots. Slides can frequently be put back-to-back in the slide holder slots.

7. Incubate in a humidified chamber (if PAP) at 37°C for several hours or room temperature overnight, until color has developed to a maximum.
8. Remove X-gal solution from each slide and rinse in excess of PBS in a slide rack for several minutes.
9. Using a cut-off Pasteur pipette, apply a small amount of **aqueous** mounting medium like [Gelvatol](#) ([Celvol](#); [Airvol](#)) over the sections and carefully lower a cover slip over the slide, taking care to avoid air bubbles and to prevent the coverslip from sliding from side to side as it is being lowered. Allow to dry flat overnight and store at room temperature indefinitely.

X-gal fixative solution

4% formaldehyde

0.5% glutaraldehyde

0.1 M Na phosphate buffer, pH 7.2 or PBS

Formaldehyde can be diluted from concentrated ampules (cleanest and safest source).

Alternatively, heat 200 ml of Na phosphate buffer with 16 g paraformaldehyde powder in a chemical fume hood with stirring until dissolved. Add 8 ml of 25% glutaraldehyde and Na phosphate buffer up to 400 ml. Store at 4°C for at least several months. *Note: Using*

formalin (37% liquid formaldehyde) instead of ampules or paraformaldehyde powder as a source of formaldehyde appears to give identical results.

X-gal staining solution

0.82 g $\text{K}_3\text{Fe}(\text{CN})_6$ (5 mM final)

1.05 g $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ (5 mM final)

1 ml of 1 M MgCl_2 (2 mM final)

PBS up to 500 ml

Store in the dark at room temperature for several months or until considerable precipitate forms.