X-gal Staining of Cells

1. Fix cells in ice cold X-gal fixative solution for 4 minutes in a chemical fume hood.

   The fixing itself can be at room temperature.

2. Rinse with PBS, let sit for 1 minute.

3. Rinse with more PBS, let sit for 10 minutes.

4. Dilute 40 mg/ml X-gal 1:40 into staining solution immediately before use. Replace PBS with diluted X-gal solution.

5. Incubate in a humidified chamber at 37˚C (cell culture incubator is fine) for one-several hours until color has developed to a maximum.

6. Remove X-gal solution and rinse in PBS for several minutes.

   Phase optics are not good for viewing X-gal stained cells because it masks the difference between lightly stained and unstained cells. A convenient way to see both stained and unstained cells is to push the phase slider part of the way in, which gives a fake Nomarski-style image. True Nomarski (D.I.C.) is also a good option if available, but if cells were grown in tissue culture plastic dishes, the plastic can interfere with the quality of the Nomarski optics.

X-gal fixative solution

4% formaldehyde
0.5% glutaraldehyde
0.1 M Na phosphate buffer, pH 7.2
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 $K_3Fe(CN)_6$ (5 mM final)
1.05 g $K_4Fe(CN)_6 \cdot 3H_2O$ (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.
Some precipitate at the bottom is normal; do not shake the solution.