X-gal Staining Soriano Lab

Fixative solution for embryos, embryo sections, and ES cells: 2% formaldehyde (from a 37% stock), and 0.2% glutaraldehyde (grade I, from a 25% stock) in PBS; make fresh for each use. Fix on ice for following times:

Age of Embryo (days)	Volume of fixative and strain (ml)	Time for fixation (min)
Cells or e7.5 – e8.5	2	15
e9.5 – e10.5	5	30
e11.5 – e12.5	10	60
e13.5 – e14.5	15	90

Volumes are suitable for a litter of up to 12 embryos.

Staining solution for embryos, embryo sections, and ES cells: 1 mg/ml X-Gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM MgCI, 0.01% sodium deoxycholate, and 0.02% Nonidet P-40 (NP-40) in PBS. This solution, without X-Gal, can be prepared in advance and stored at room temperature in the dark. Add X-Gal from a stock solution just before use.

X-Gal (5 -Bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) stock: 40 mg/ml in dimethylformamide; store at - 20 degrees in small aliquots and ensure that X-Gal is well dissolved before use otherwise crystals may form in the staining solution.

Please note that for larger embryos (E11.5 and above) and adult tissues, there is very inefficient penetration of the X-Gal stain. According to the tissue, penetration may be less than 1 mm. In such cases, we have found the following method to be advantageous:

1. Fix the embryo or tissue only for a short time.
2. Make 500 um-1mm sections using a vibratome.
3. Refix the tissue.
4. Stain with X-Gal as above.

Using such a protocol, we obtain uniform staining of tissues, for instance with ROSA26.