

Protocol for Xgal staining of Lung specimens:

3/1/2000 modified 5/2001 Kotton Lab

Prepare (see Xgal solution protocol for details if needed)

fresh 0.1% Xgal (or Bluo gal) solution (filtered through .2-.4 micron filter):

5mM K-Ferricyanide

5mM K-Ferrocyanide

2mM MgCl₂

in PBS, pH =7.4, Filter the final solution (0.45 micron filter) to avoid any precipitate.

Keep this wrapped in foil in fridge until use - use same day. (NB: lungs have some endogenous B-gal, particularly in alveolar macrophages and large airway epithelium: to favor X-gal staining of bacterial rather than endogenous mammalian B-gal, the X-gal solution can be made in buffer containing Tris (24mM)/NaCl (137mM)/ pH=7.4, rather than PBS).

Also prepare 0.25% glutaraldehyde with 2mM MgCl₂ in PBS, pH=7.4

Protocol:

Harvest Mouse by CO₂ asphyxiation

Dissect mouse , transecting abdominal aorta, exposing lungs, trachea

Optional: irrigate right ventricle with 3cc of 0.25% glutaraldehyde/2mM MgCl₂

Place suture behind trachea

Insert into trachea a blunt end canula on 3cc syringe with 0.25% gluta/2mM Mgcl₂

Tie canula in place with suture around trachea

Inflate lungs with 0.25% gluta/2mM MgCl₂ at 20cm H₂O pressure (=about 1.5 cc)

Use a dropper to wet outside of lungs with some glutaraldehyde.

Let sit to fix for 10-15 minutes

Suck out gluta deflating lungs

Change syringe to PBS syringe leaving canula in place

Aspirate out air and then inflate and deflate lungs with PBS 2-3 times

Change syringe to Xgal (or Bluo Gal) syringe, suck out air and then inflate lungs with Xgal solution

Remove canula and tie suture tight to keep solution in lungs

Dissect out lungs and place in 50cc conical with 10cc Xgal solution (or Bluo Gal) at room temp overnight (6 to 18 hrs)

Wash with PBS twice, Dissect out lobes, wash in PBS

Post fix overnight in fresh 4% paraformaldehyde in PBS, pH=7.4 and embed in paraffin by standard techniques.

Equipment needed:

dissecting tools/board 70% ETOH to clean mouse

Sutures Filtered Xgal or Bluo Gal solution (see above)

0.25% glutaraldehyde in PBS with 2mM MgCl₂

50cc Conical Tube with PBS 3x3cc syringes

Blunt end canula (can be made by breaking the sharp tip off a 22 gauge needle)

Timer

50cc conical for each lung specimen

4% paraformaldehyde