Heather LaMarca

X-gal Staining of Mammary Glands

1. Remove glands and fix in cold 4% PFA for 1 hour at RT.

2. Briefly rinse glands in 1X PBS.

3. Wash glands 3X for 30′ each with Wash Buffer at RT with rocking.

4. Pre-incubate glands in Xgal Solution WITHOUT Xgal for 1 hour at 37°C.

5. Add 1mg/ml Xgal to solution and incubate O/N at 37°C.

6. Dehydrate:

70% EtOH	1 hour
95% EtOH	1 hour
100% EtOH	1 hour
100% EtOH	1 hour
Histoclear	1 hour
Histoclear	1 hour
Histoclear	overnight

7. Embed in paraffin and section tissue.

Can counterstain with Carmine Alum or Neutral Red prior to dehydration. After sectioning, can counterstain with hematoxylin or nuclear fast red.

Note: Histoclear can interfere with immunostaining of subsequent sections. If immunostaining is desired, clear glands in 100% EtOH for several days to visualize ducts by whole mounts. Process for paraffin embedding as usual. Xylene can be used, but only for short periods of time (1 hour).

Wash Buffer: 0.2% NP-40, 0.01% NaDOC, 2mM $MgCl_2$ in 1X PBS

400 μl	1M MgCl
400 μl	NP-40
200 μl	10% Sodium Deoxycholate
199 ml	1X PBS
200 ml	

X-gal Solution: 25mM $K_3Fe(CN_6)$, 25mM $K_4Fe(CN_6)\bullet 3H_20$, 1mg/ml Xgal in Wash Buffer

25 ml	100 mM 25mM $K_3Fe(CN_6)$
25 ml	100mM $K_4Fe(CN_6)\bullet 3H_20$
2.5 ml	40mg/ml Xgal
49 ml	Wash Buffer
99 ml	

X-gal (InVitrogen #15520-034): Resuspend 100 mg vial in 2.5 ml of DMF → 40 mg/ml stock