



X-Gal Staining of Adult Bone Samples

Protocol:

1. Fix fresh bone samples with 0.2% glutaraldehyde at 4°C 5 days (adult bones).
2. Wash twice with PBS for 15 minutes.
3. Decalcify in 14% EDTA (PH 7.2-7.4) at 4°C. Change daily for 10 days.
4. Wash twice with PBS for 15 minutes.
5. Place samples in 15% sucrose (diluted in PBS) overnight at 4°C, then 30% sucrose (diluted in PBS) at 4°C overnight.
6. Place in O.C.T media at room temperature for at least 30 min. prior to freezing.
7. Cryosection – cut frozen section 8-10 um and place slides at -20°C until needed.
8. Wash slides in wash buffer twice with PBS for 15 minutes.
9. Stain in X-Gal staining solution for 4 hours to overnight.
10. Wash twice with PBS for 15 minutes.
11. Counter stain with Nuclear Fast Red for 5 min.



Solutions:

Fixing solution (0.2% glutaraldehyde)

2% NP40 0.5 ml

0.5M EDTA 0.5 ml

25% glutaraldehyde 0.4 ml

1M MgCl₂ 0.1 ml

PBS total to 50 ml

Washing buffer

2% NP40 2.0 ml

1M MgCl₂ 0.4 ml

1% Na Deoxycholate 2.0 ml

PBS total to 200 ml

Staining Solution

50 mg/ml X-Gal in Dimethylformamide 1.0 ml

Potassium ferrocyanide 0.106 g

Potassium ferricyanide 0.082 g

1M Tris PH 7.4 1.0 ml

Washing buffer total to 50 ml

5-Bromo-4-chloro-3-indolyl- β -D-galactopyranoside B4252-500mg Sigma-Aldrich 076K1368

X-gal 1g Gold BioTechnology (GBD) #X4281C