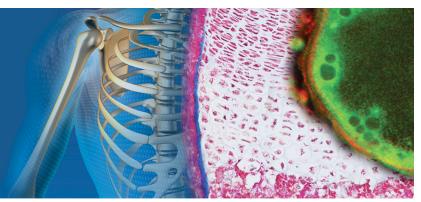
CENTER for MUSCULOSKELETAL Research



βGal Staining Protocol (Frozen Sections)

- 1. Remove frozen sections from the -20°C freezer and circle sections with pap pen.
- 2. Fix sections in 0.2% gluteradehyde (8 µl 25% gluteraldehyde in 1ml 1X PBS) for 10 min at 4°C.
- 3. Wash slides 3 times in LacZ Wash for 5 min each at room temperature.

LacZ Wash: Amount of stock 1.5 ml of 1M MgCl₂

7.5 ml of 1% Na Deoxycholate 7.5 ml of 2% NP-40 733.5 ml of 1X PBS

Final Conc. 2mM MgCl₂ 0.01% Na Deoxycholate 0.02% Nonidet P-40

Notes: Mix and remove 10 ml for LacZ staining solution. Use the rest for the 3 LacZ washes in 200 ml glass histology jars.

4. With the 10 ml of LacZ wash buffer make the LacZ staining solution.

LacZ Stain:	Amount of stock	Final Conc.
	100 μl of 0.5M K ₃ Fe(CN) ₆	5mM K ₃ Fe(CN) ₆
	100 μl of 0.5M K ₄ Fe(CN) ₆	5mM K ₄ Fe(CN) ₆
	100 μl of 50 mg/ml X-Gal	0.5 mg/ml X-Gal

Notes: Mix and wrap in aluminum foil to keep protected from light.

- 5. Add enough LacZ stain to cover each section. Slides should be placed in a humidified chamber and protected from light at 37°C for 4hrs to overnight.
- 6. When color reaction is finished rinse in 1X PBS, rinse in Milli-Q water, and then counterstain with Nuclear Fast Red for 5 minutes.
- 7. Dehydrate in 75% EtOH, 100% EtOH, clear in Xylenes, and coverslip.





MEDICINE of THE HIGHEST ORDER