



## $\beta$ Gal Staining Protocol (Frozen Sections)

1. Remove frozen sections from the  $-20^{\circ}\text{C}$  freezer and circle sections with pap pen.
2. Fix sections in 0.2% glutaraldehyde (8  $\mu\text{l}$  25% glutaraldehyde in 1ml 1X PBS) for 10 min at  $4^{\circ}\text{C}$ .
3. Wash slides 3 times in LacZ Wash for 5 min each at room temperature.

<b>LacZ Wash:</b>	<u>Amount of stock</u>	<u>Final Conc.</u>
	1.5 ml of 1M $\text{MgCl}_2$	2mM $\text{MgCl}_2$
	7.5 ml of 1% Na Deoxycholate	0.01% Na Deoxycholate
	7.5 ml of 2% NP-40	0.02% Nonidet P-40
	733.5 ml of 1X PBS	

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.

<b>LacZ Stain:</b>	<u>Amount of stock</u>	<u>Final Conc.</u>
	100 $\mu\text{l}$ of 0.5M $\text{K}_3\text{Fe}(\text{CN})_6$	5mM $\text{K}_3\text{Fe}(\text{CN})_6$
	100 $\mu\text{l}$ of 0.5M $\text{K}_4\text{Fe}(\text{CN})_6$	5mM $\text{K}_4\text{Fe}(\text{CN})_6$
	100 $\mu\text{l}$ 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  $37^{\circ}\text{C}$  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.

