

# PATHOLOGY CORE

## X-gal Staining Protocol

### Solutions:

#### 40x X-gal stock:

40mg/ml X-gal (-20Fz) in DMSO  
-Store at -20°C

#### Reaction Buffer:

20mM potassium ferrocyanide ( $K_4Fe$ ) = 8.45g/L  
20mM potassium ferricyanide ( $K_3Fe$ ) = 6.58 g/L  
2mM  $MgCl_2$  (= 2:1000 from 1 M stock)  
in PBS pH 7.8 (check pH!!!)  
-Store at 4°C

#### Final X-gal Solution:

Dilute X-gal stock 1/40 in prewarmed (37°C) reaction buffer (prepare before staining)

### X-Gal staining of cryosections:

- Cut Cryosections
- Air Dry slides
- Fix in 0.5% glutaraldehyde in PBS for 10min at 4°C  
(do not use paraformaldehyde)
- Wash in PBS 3X 5 min
- Stain in final X-gal solution at 37°C until color appears (0.5-24hrs usually overnight)
- Wash in PBS
- Counter-stain with Nuclear Fast Red
- Dehydrate and Cover-slip