

# **β-gal Staining Protocol**

## **Materials:**

Potassium ferricyanide, crystalline (Fisher Scientific #P232-500)  
Potassium ferrocyanide, trihydrate (Fisher Scientific #P236-500)  
Magnesium chloroxide (Fisher Scientific #M33-500)  
1×PBS  
X-gal reagent (Pierce #34050ZZ)  
DMSO, dimethylsulfoxide  
2% formaldehyde + 0.2% glutaraldehyde in 1× PBS  
Pap Pen (Electron Microscopy Sciences #22303)  
Eosin Y  
Cytoseal 60 (VWR # 48212-187)

## **Solutions:**

### **1) Solution A**

5 mM potassium ferricyanide, crystalline  
5 mM potassium ferrocyanide, trihydrate  
2 mM magnesium chloroxide

### **2) X-gal Stock Solution (30×)**

40 mg/ml in DMSO (100 mg in 2.5 ml DMSO)

### **3) Final X-gal Solution**

Dilute X-gal stock solution 1:30 in Solution A.  
(first warm Solution A to 37° C to prevent precipitation of X-gal)

## **Staining Protocol:**

Start with fixed frozen 10-μm tissue sections on pap-penned slides

- 1) Allow slides to completely dry for 1 hour.
  - 2) Fix tissue with 2% formaldehyde + 0.2% glutaraldehyde for 5 minutes.
  - 3) Rinse with 1× PBS.
  - 4) Incubate slides in X-gal solution in humidity chamber at 37°C overnight.
  - 5) Rinse with 1× PBS
  - 6) Immerse slides in 100% ethanol until all precipitate dissolves (1–3 hours).
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7) Place slides in:

|              |            |
|--------------|------------|
| Eosin Y      | 25 seconds |
| 95% Ethanol  | 1 minute   |
| 100% Ethanol | 1 minute   |
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| Xylene       | 2 minutes  |
| Xylene       | 10 dips    |
| Xylene       | Coverslip  |

8) Coverslip slides using Cytoseal 60.