Conklin Lab
Baygenomics Component 4

X-gal Staining Protocol

(Bruce Conklin and David Sanan, Gladstone/UCSF)

More information from the Conklin Lab is available at www.conklinlab.org

Materials:

Potassium Ferricyanide Crystalline (Fisher Scientific #P232-500
Potassium Ferricyanide Trihydrate (Fisher Scientific #P236-500)
Magnesium Chloride (Fisher Scientific #M33-500)
1× PBS
X-gal (Boehringer Mannheim #745-740)
DMSO
2% Formaldehyde 0.2% Gluteraldehyde in 1× PBS
Superfrost/ Plus Microscope Slides (Fisher #12-550-15)
Pap pen (Electron Microscopy Sciences #22303)
Nuclear Fast Red (also called Kernechtrot)
Gel Mount (Biomeda, M01)

Solutions:

1) Solution A:
5mM Potassium Ferricyanide Crystalline
5mM Potassium Ferricyanide Trihydrate
2mM Magnesium Chloride
in 1× PBS
(stored at 4^{o}C, protected from light, then warm to 37^{o}C prior to using)

2) X-gal Stock Solution (40×):
40 mg/ml in DMSO (100 mg in 2.5 ml DMSO)
(store at -20^{o}C, protected from light)

3) Final X-gal Solution:
Dilute X-gal stock solution 1:40 in Solution A.
(first warm Solution A to 37^{o}C to prevent precipitation of X-gal)

4) Formalin/Glutaraldehyde fixative:
12.3 ml distilled water
10.0 ml 1% glutaraldehyde
2.7 ml formaldehyde stock (37%)
25.0 ml × 2 saline buffer

Staining Protocol:

1. Cut 10 micrometers cryostat sections onto pap-penned slides from fresh-frozen tissues. Immerse immediately into cold formaldehyde/gluteraldehyde 5 minutes, then rinse in dH20 for 60 seconds.
2. Let section dry completely onto slide
3. Rinse with 1× PBS
4. Apply Final X-gal Solution to sections and incubate at 37^{o}C; for 30 minutes to 24 hours (Check sections under microscope every 2 hours for development of blue staining)
5. Rinse with 1× PBS
6. Wash 2 × 2 minutes with deionized H2O.
7. Counterstain 3 minutes with Nuclear Fast Red
8. Wash as in step 6, then cover slip with Gel Mount