X-Gal Staining of Frozen Sections

Sources: a blend of http://www.ihcworld.com/_protocols/special_stains/x_gal.htm and https://www.jax.org/research-and-faculty/resources/cre-repository/fresh-frozen-slide-staining-protocol and Matt Warmann (Lubricin Restoration in a Mouse Model of Congenital Deficiency, *Arthritis and Rheumatology* 2015)

Reagents to Prepare:

- X-gal staining solution (30 mL):
  - 0.02% IPEGAL (Sigma) - 6 uL
  - 5 mM Potassium Ferricyanide - 49.35 mg
  - 5 mM Potassium Ferrocyanide - 63.3 mg
  - 2 mM $MgCl_2$ - 60 uL 1M stock
    - All diluted in PBS
- 25 mg/mL X-gal in Dimethylformamide (DMF) stock
  - Make 2.5 mL DMF with 62.5 mg X-gal
- 2 mM MgCl2 and 0.02% IPEGAL in PBS (5 mL)
  - 10 uL 1M $MgCl_2$ stock
  - 10 uL 1:10 dilution of stock
- 0.2% glutaraldehyde, 5 mM EDTA, and 2 mM MgCl2 in PBS (1 mL volume)
- 4% PFA in PBS (standard procedure)

Protocol:

1. Cut 10 um sections of fresh frozen tissue and store at -20C or -80C until fix/stain (5 um sections for knee cartilage)
2. Pre-warm the X-gal staining solution to 55C. Must be dark.
3. Immediately prior to staining, fix with Glutaraldehyde fixative solution for 5 min.
4. Prepare the working X-Gal solution by adding 2 mg/mL X-gal to 55C X-gal staining buffer, place solution in 37C incubator at 100 rpm. Buffer must be 37C before tissue slides are added.
5. Wash slides 3X in 2 mM MgCl2 and 0.02% IPEGAL in PBS 5 min each wash, rinse in distilled water
6. Incubate slides in X-gal working solution at 37C for 24 hours (use humidified chamber to prevent drying) in the dark shaking.
7. Post-fix slides in 4% PFA for 10 min
8. Rinse sections in PBS 2x5 min
9. Rinse sections in distilled water briefly
10. Counter stain with Nuclear Fast Red 5 min, rinse in distilled water, and wash in distilled water for 2 min
11. Let slides dry, mount with Mounting media, and coverslip