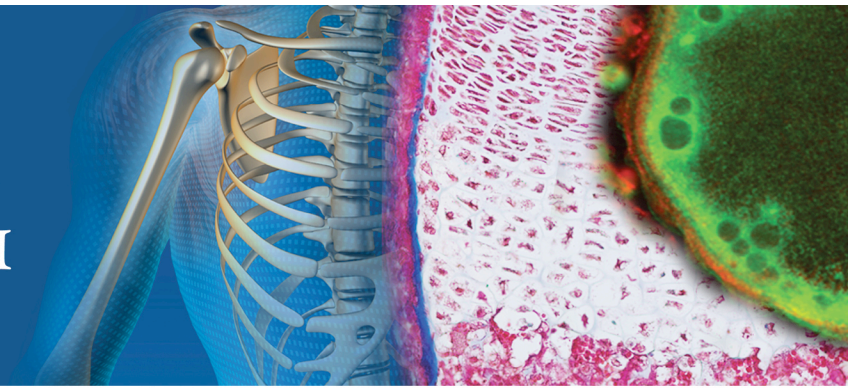

Frozen Sections Protocol for Embryos/Adults

1. Dissect embryos (skin embryos older than E15.5) or postnatal limbs in cold PBS.

2. Briefly fix embryos (or postnatal limbs that have been skinned and cleared of most muscle tissue) in 4% Paraformaldehyde (PFA), pH 7.1-7.4.
    a. Embryos or soft tissue fix for 30 min.
    b. P0-P21 pups fix for 1 hour.
    c. Adult limbs fix for 1.5 hours.

3. Wash 3 times in 1XPBS for 5-10 min each at 4°C (rocking).

4. Decal embryos in 14% EDTA, pH 7.5 (rocking). Do **NOT** use ImmunoCal!
    a. Embryos older than E15.5 decal for 24-48 hours.
    b. P1-P4 decal for 3 days (change daily).
    c. Less than 3 week-old decal for 5-7 days (change daily).
    d. Adult limbs decal for 10 days (change daily).

5. Wash 3 times in 1XPBS for 5-10 min each at 4°C (rocking).

6. Wash embryos (or limbs) in 15% sucrose (1X PBS) at 4°C until they sink to the bottom of the vial (Overnight is best). Change to 30% sucrose (1X PBS) and leave overnight at 4°C (rocking).

7. Transfer tissue to be sectioned into OTC at room temperature for 10 min before freezing on dry ice. Freeze on dry ice for 10-15 minutes before sectioning.

8. Section at 8-10μm for immunohistochemistry or β-galactosidase assays.

Notes: When sectioning place slide box (where fresh cut sections will be stored) on a block of dry ice to keep slides cold. When finished sectioning, store sections in -20°C freezer. β-galactosidase assays can be performed on sections a few months following the original date of sectioning.