Penn Center for Musculoskeletal Disorders Histology Core

**Protocol:** Alcian blue and picrosirius red staining

**Materials**
- Alcian blue pH2.5 stain
  - 1.0 g Alcian blue 8GX + 97 ml distilled water (heat to dissolve), + 3 ml glacial acetic acid. Filter before first use.
  - **Application Note:** This method stains all GAGs. To stain highly sulfated GAGs only, reduce pH to 1.0.
- Acidified water
  - 0.5% aqueous acetic acid
- Picrosirius red staining solution
  - 0.1 g sirius red (also called direct red) in 100 ml saturated picric acid.
  - **Safety note:** picric acid is a percussive explosive when dry. Always clean residue from surfaces.
- Mounting medium
- Citrisolve (or xylene)
- Ethanol

**Deparaffinize and Rehydrate** (for paraffin sections)
1. Citrisolve 5 mins
2. Citrisolve 5 mins
3. 100% ethanol 5 mins
4. 100% ethanol 5 mins
5. 95% ethanol 5 mins
6. 70% ethanol 5 mins
7. Deionized water 5 mins

**Staining**
1. Stain in Alcian blue for 15 mins
2. Rinse in running tap water for 10 mins
3. Stain in picrosirius red for 45 mins
4. Rinse in acidified water for 5 mins
Dehydration and Mounting
1. 95% alcohol 2 mins
2. 100% alcohol 2 mins
3. 100% alcohol 2 mins
4. Citrisolve 2 mins
5. Citrisolve 2 mins
6. Coverslip

Application Notes:
• For picrosirius red only, skip staining steps 1 and 2
• For Alcian blue only, skip steps 3 and 4, and go through 70% ethanol before 95% during dehydration

Expected Results:
• Glycosaminoglycans: blue
• Collagens: red

Suggested Controls:
• Cartilage or intervertebral disc