

Penn Center for Musculoskeletal Disorders Histology Core

Protocol: Alcian blue and picosirius 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
- Picosirius red staining solution
 - 0.1 g sirius red (also called direct red) in 100ml 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 picosirius 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 picosirius 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