

Penn Center for Musculoskeletal Disorders Histology Core

Protocol: Safranin O and fast green staining

Materials

- Safranin O solution (0.2% aqueous)
- Fast green solution (0.02% aqueous)
- Acetic acid solution (1% aqueous)
- 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 fast green solution for 3 mins
2. Submerge in acetic acid solution for 10 secs
3. Stain in safranin O solution for 10 mins
4. Rinse in tap water for 5 mins

Dehydration and Mounting

1. 70% ethanol 2 mins
2. 95% alcohol 2 mins
3. 100% alcohol 2 mins
4. 100% alcohol 2 mins
5. Citrisolve 5 mins

6. Coverslip

Expected Results:

- Glycosaminoglycan-rich tissue: red/orange
- Other connective tissue: bluish green

Suggested Controls:

- Cartilage or intervertebral disc

References

- Bryan J.H.D. (1954). *Differential Staining with a Mixture of Safranin and Fast Green FCF*, Stain Technology, 30(4):153-157