

Von Kossa – MacNeal's Tetrachrome (Thin Acrylosin Sections)

N.B. Slides can be deplastified using acetone or xylenes;

1. Remove plastic film
2. Place slides in 2 exchanges of warm xylenes @ 50C for 30 min each; dip and dunk after 15 min
3. Place slides in cool xylenes @ RT for 30 min; dip and dunk after 15 min
4. Re-hydrate through graded series of ethanol:
 - a. 100% EtOH 5+ min
 - b. 95% EtOH 5+ min
 - c. 70% EtOH 5+ min
 - d. dH₂O 5+ min (can be held here for at least 2-3 hours)
5. Stain in Silver Nitrate; **KEEP OUT OF LIGHT** (*Dorn & Hart Microedge*) 5 min
6. Rinse in dH₂O x 3 exchanges; **KEEP OUT OF LIGHT** 1+ min (each)
7. Develop in Sodium Carbonate-Formaldehyde (*Dorn & Hart Microedge*) 2 min
8. Rinse in dH₂O x 2 exchanges 1+ min (each)
9. Clear in Farmer's Diminisher (*Dorn & Hart Microedge*) 30 sec
* 210 ml Farmer's Diminisher Solution + 1 g Farmer's Diminisher Activator
10. Wash in gently running tap water 15 min
11. Rinse in dH₂O 1+ min
12. Stain in MacNeal's Tetrachrome (*Dorn & Hart Microedge*) 5+ min
13. Rinse in dH₂O x 3 exchanges 1+ min (each)
14. Dehydrate, clear, and mount
 - a. 70% EtOH 1+ min
 - b. 100% EtOH 1+ min
 - c. Clear in xylenes 3 min
 - d. Clear in xylenes 3+ min
 - e. Coverslip with Eukitt (*Dorn & Hart Microedge*)