

Von Kossa Calcium Stain - Technical Memo

SOLUTIONS:	250 ml	500 ml	1 Liter
Silver Nitrate 5%, Aqueous	Part 13805A	Part 13805B	
Sodium Thiosulfate 5%, Aqueous		Part 1389A	Part 1389B
Nuclear Fast Red Stain, Kernechtrot	Part 1255A	Part 1255C	Part 1255B

Additionally Needed:

Calcium Control Slides	Part 4100
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Von Kossa Calcium Stain is an indirect staining method for the demonstration of calcium or calcium salt in tissue sections. This technique is not specific for calcium and other reducing substances, such as formalin pigment and melanin, will also give a positive reaction.

METHOD:

Fixation: Alcohol or Formalin 10%, Phosphate Buffered (Part 1090)

- Avoid fixatives and solutions containing calcium

Technique: Paraffin sections cut at 5 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. All glassware/plasticware must be acid cleaned prior to use.
 - a. See Procedure Notes #1 and #2.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Notes #3 and #4.
3. Place slides in Silver Nitrate 5%, Aqueous according to the following timings and conditions.
 - a. Direct sunlight or ultraviolet light for 10-30 minutes.
 - b. In front of a 60-100 watt light bulb for 1 hour or longer.
 - c. See Procedure Note #5.
4. Check slides periodically and remove from light source when control slide shows black-brown deposits macroscopically.
5. Rinse well in several changes of distilled water.
6. Place slides in Sodium Thiosulfate 5%, Aqueous for 2 minutes.
7. Rinse well in several changes of distilled water.
8. Counterstain in Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
 - a. Shake solution well before use; do not filter.
9. Rinse well in distilled water.
 - a. See Procedure Note #6.
10. Dehydrate in two changes each of 95% and 100% ethyl alcohol; 10 dips each. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Calcium salts	Black to brown/black
Nuclei	Red
Cytoplasm	Light pink

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water. Cleaning glassware with bleach is not equivalent to acid washing.
2. Plastic (5500), plastic-tipped (5502, 5503), or paraffin coated metal forceps must be used with any silver solution to prevent precipitation of silver salts. No metals of any kind should be in contact with any silver solution.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining procedure.
5. Direct sunlight is the preferred reaction method. If the procedure is carried out in minimal sunlight or subdued light, increased incubation time will be necessary.
6. Wash well after Nuclear Fast Red, Kernechtrot to avoid cloudiness in dehydration steps.
7. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 269-270.
2. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 176-177.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 226-227.
4. Modifications developed by Newcomer Supply Laboratory.