

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Elastic Stain Instructions for Use

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

No special requirements; formalin fixation is adequate.

Quality Control

A cross section of aorta or large artery should be used.

Technical Procedure

Working Elastic Stain Solution (Prepare Fresh)

Alcoholic Hematoxylin Solution..... 20ml
 Ferric Chloride Solution.....8ml
 Weigert's Iodine Solution..... 8ml
 Mix Well

Working Differentiating Solution (Prepare Fresh)

Ferric Chloride Solution.....8ml
 Deionized or Distilled Water.....32ml
 Mix Well

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Stain sections in Working Elastic Stain Solution for 15 minutes to 1 hour.
3. Rinse excess stain off sections in running tap water for 1 minute.
4. Decolorize sections in Working Differentiating Solution and agitate gently.
5. Rinse sections in tap water.

Note: Endpoint should be determined by microscopic evaluation. If over-differentiated, return sections to Working Elastic Stain Solution step.

6. Place sections in Sodium Thiosulfate Solution for 1 minute.
7. Rinse sections in deionized water for 30 seconds.
8. Stain sections in Van Gieson Stain Solution for 1 minute.
9. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
10. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Caution: Thermo Scientific Mounting Medium (Cat# 4111 and 4112) and Thermo Scientific ClearVue Mountant (Cat# 4211 and 4212) will leach picric acid from Van Gieson Stain Solution. Use alternative mounting medium.

Results

Elastic Fibers – Black
 Nuclei – Black
 Collagen – Red
 Other Tissue Structures – Yellow

Discussion

All staining reagents should be stored at room temperature. The Elastic staining reagents are for "In Vitro" use only. Refer to the Material Safety Data Sheet for Health and Safety Information. All reagents are stable and should not form precipitants under ordinary storage parameters. It is recommended that the Elastic Stain Solution be discarded after procedure is complete. It is also recommended that the Working Differentiating Solution and Sodium Thiosulfate Solution be discarded after each use. The Van Gieson Stain Solution can be filtered and reused. These stains should not be diluted and are ready-for-use. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

It is not necessary to remove mercury pigments from tissue fixed in the various mercury fixatives, as they will be removed during the staining process. During microscopic examination, elastic fibers should be black and sharply defined with a grey background. The Van Gieson Stain Solution will act to further differentiate the elastic fibers, so it is generally best to slightly under-differentiate.

Probable Mode of Action

In this technique the tissue is over-stained with the dye-lake comprised of hematoxylin, ferric chloride, and iodine. The tissue elements are differentiated by using excess ferric chloride to break the dye-lake complex. Since elastic fibers have the strongest affinity for the hematoxylin-ferric chloride-iodine dye lake, it is the tissue element that will maintain its dye lake bond the longest. This is a regressive method, and care should be taken to avoid over-differentiation.

References

1. Bancroft, J.D. and Stevens, A. Theory and Practice of Histological Techniques. Churchill Livingstone, New York, NY, 1977.
2. Sheehan, D.C. and Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Mosby, St. Louis, MO, 1980.
3. Thompson, C.C. Selected Histochemical and Histopathological Methods. Springfield, IL, 1966.
4. Lillie, R.D., H.J. Conn's Biological Stains. Williams & Wilkins, Baltimore, MD, 1972.
5. Carson, F.L. Histotechnology: A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago. 1997.

Order Information

Product	Size	Qty.	REF
Elastic Stain Kit	1 Kit	1	87017
Alcoholic Hematoxylin Solution	250 ml	1	88007
Ferric Chloride Solution	125 ml	1	88008
Weigert's Iodine Solution	125 ml	1	88009
Sodium Thiosulfate Solution	500 ml	1	88000
Van Gieson Stain Solution	250 ml	1	88011

