

Thermo Scientific™ Richard-Allan Scientific™ Chromaview™ – Advanced Testing Gomori's Trichrome Stain – Blue Collagen Instructions for Use

For in vitro diagnostic use.

For use as a kit in special staining techniques.

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

No special requirements; formalin fixation is adequate. Tissue fixed in Bouin's Fluid does not require Bouin's pretreatment as outlined in Step 2.

Quality Control

A section of uterus, small intestine or appendix should be used.

Technical Procedure

Working Weigert's Iron Hematoxylin

Mix equal quantities of Part A and Part B.

Solution will last up to 10 days at room temperature.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Place sections in Bouin's Fluid at 56° C for 1 hour.
3. Rinse sections in running tap water for 3-5 minutes until yellow color is removed.
4. Place sections in Working Weigert's Iron Hematoxylin Stain for 10 minutes.
5. Rinse sections in running tap water for 5-10 minutes.
6. Stain sections in Trichrome Stain for 15 minutes.
7. Place sections in 1% Acetic Acid Solution for 1 minute.
8. Rinse sections in deionized water for 30 seconds.
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.

Microwave Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Place sections in 40 mL of Bouin's Fluid in a plastic coplin jar with lid applied loosely. Microwave on high for 30 seconds. Let stand 5 minutes.
3. Rinse sections in tap water for 3-5 minutes until yellow color is removed.
4. Place sections in Working Weigert's Iron Hematoxylin Stain for 10 minutes.
5. Rinse sections in tap water for 5-10 minutes.
6. Stain sections in Trichrome Stain for 15 minutes.
7. Place sections in 1% Acetic Acid Solution for 1 minute.
8. Rinse sections in deionized water for 30 minutes.
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.

Results

Nuclei – Black
Cytoplasm and Muscle Fibers – Red
Collagen – Blue

Discussion

All staining reagents should be stored at room temperature. The Gomori's Trichrome staining reagents are for "In Vitro" use only. Refer to the 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 Bouin's Solution and 1% Acetic Acid Solution be discarded after use. The Gomori's Trichrome Stain – Blue Collagen can be filtered and reused. The Working Weigert's Iron Hematoxylin can be reused for up to 10 days depending on the volume of slides. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

More intense staining is achieved with the room temperature procedure. Exposure to hot Bouin's Fluid may cause sections to fall off slides and will increase exposure to hazardous fumes. It is recommended to use Thermo Scientific Poly-L-Lysine coated slides or Thermo Scientific Bond-Rite Adhesive Slides. Use caution when microwaving Bouin's Fluid. Use with adequate ventilation or a fume hood. The microwave protocol was developed using a 1200 watt microwave oven. Microwave frequencies vary from model to model. It may be necessary to adjust power levels or times to achieve desired results.

Probable Mode of Action

In this method the cytoplasmic stain and the collagen stain are combined into one solution along with phosphotungstic-phosphomolybdic and glacial acetic acids. Muscle cells and cytoplasm favor the red dyes, and this is facilitated by the phosphotungstic acid. Collagen fibers take up the tungstate ion, allowing for the blue dye to selectively bind.

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
Gomori Trichrome Kit (Blue Collagen)	1 Kit	1	87020
Weigert's Iron Hematoxylin – Part A	500 mL	1	88028
Weigert's Iron Hematoxylin – Part B	500 mL	1	88029
Gomori Trichrome Stain Solution, Blue Collagen		500 mL	1
			88030
Bouin's Fluid	500 mL	1	88038
1% Acetic Acid Solution	500 mL	1	88039

Anatomical Pathology



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Richard-Allan Scientific
Subsidiary of Thermo Fisher Scientific
4481 Campus Drive, Kalamazoo, MI 49008
Phone: 1 (800) 522-7270
Phone: + 1 (269) 544-5600
www.thermoscientific.com/pathology



Thermo Shandon Limited
Subsidiary of Thermo Fisher Scientific
Tudor Road, Manor Park,
Runcorn, Cheshire WA7 1TA, UK
Phone: +44 (0) 1928 534 050
Fax: + 44 (0) 1928 534 049



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