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Thermo Scientific™ Richard-Allan Scientific™ Chromaview™ – Advanced Testing Masson Trichrome Stain 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 Solution
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 deionized 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 deionized water for 5-10 minutes.
6. Stain sections in Biebrich Scarlet-Acid Fuchsin Solution for 5-10 minutes to achieve the desired intensity.
7. Rinse sections in deionized water for 30 seconds.
8. Place sections in Phosphotungstic-Phosphomolybdic Acid Solution for 5 minutes.
9. Stain sections in Aniline Blue Stain Solution for 5-10 minutes to achieve the desired intensity.
10. Place sections in 1% Acetic Acid Solution for 1 minute.
11. Rinse sections in deionized water for 30 seconds.
12. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
13. 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 deionized 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 deionized water for 5-10 minutes.
6. Stain sections in Biebrich Scarlet-Acid Fuchsin Solution for 5-10 minutes to achieve the desired intensity.
7. Rinse sections in deionized water for 30 seconds.
8. Place sections in Phosphotungstic-Phosphomolybdic Acid Solution for 5 minutes.
9. Stain sections in Aniline Blue Stain Solution for 5-10 minutes to achieve the desired intensity.
10. Place sections in 1% Acetic Acid Solution for 1 minute.
11. Rinse sections in deionized water for 30 seconds.
12. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
13. 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 Masson 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 Phosphotungstic-Phosphomolybdic Acid Solution be discarded after each use. The Bouin's Fluid and 1% Acetic Acid Solution should also be discarded after each use. The Biebrich Scarlet-Acid Fuchsin Solution and Aniline Blue Stain Solution can be filtered and reused. These stains should not be diluted and are ready for use. Also, 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. For less intense staining, use a 5-minute stain for each staining reagent. Use caution when microwaving Bouin's Fluid. Overheating will also cause sections to fall off from slides and will increase exposure to hazardous fumes. Therefore, it is recommended to use Poly-L-Lysine coated slides or Bond-Rite Adhesive Slides. Use with adequate ventilation or a fume hood. The microwave protocol was developed on 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

All of the acidophilic tissue elements first stain with Biebrich Scarlet-Acid Fuchsin. This includes the cytoplasm of cells, muscle tissue, and collagen. Phosphotunsic-phosphomolybdic acid treatment differentiates between collagen and muscle. It causes the Biebrich Scarlet Acid Fuchsin to be removed from the collagen but not the cytoplasm of muscle cells. The Phosphotunsic-phosphomolybdic acid is also thought to play a role as a link between the collagen and the subsequent aniline blue dye.

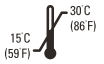
References

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
Masson Trichrome Kit	1 Kit	1	87019
Biebrich Scarlet-Acid Fuchsin Solution	250 mL	1	88019
Phosphotungstic-Phosphomolybdic Acid Solution		250 mL	1 88020
Aniline Blue Stain Solution	250 mL	1	88022
Weigert's Iron Hematoxylin – Part A	500 mL	1	88028
Weigert's Iron Hematoxylin – Part B	500 mL	1	88029
Bouin's Fluid	500 mL	1	88038
1% Acetic Acid Solution	500 mL	1	88039

Anatomical Pathology



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