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

For in vitro diagnostic use.
For use as a kit in special stain techniques.

Technical Discussion

Microtomy
Cut sections at 4-6 microns.

Fixation
No special requirements; formalin fixation is adequate.

Quality Control
A section containing ferric iron should be used.

Technical Procedure

Working Iron Solution
Potassium Ferrocyanide Solution.....20 mL
Hydrochloric Acid Solution.....20 mL
Mix Well
Prepare just before use, using clean glassware.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
   Note: Air-dried bone marrow or blood films may be fixed in alcoholic formalin or absolute methanol for 10 minutes.
2. Rinse sections in deionized water for 1 minute.
3. Stain sections in freshly prepared Working Iron Solution for 30 minutes at room temperature.
4. Rinse sections in deionized water for 1 minute.
5. Counterstain sections in Nuclear Red Thiazin Stain Solution for 30 seconds to 1 minute to achieve desired contrast.
6. Rinse sections in deionized water for 30 seconds.
7. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
8. 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 a plastic coplin jar containing freshly prepared Working Iron Solution. Loosely cover with lid.
3. Microwave jar at 70% power for 45 seconds.
4. Rinse sections in deionized water for 1 minute.
5. Counterstain sections in Nuclear Red Thiazin Stain Solution for 30 seconds to 1 minute to achieve desired contrast.
6. Rinse sections in deionized water for 1 minute.
7. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
8. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Iron Pigments – Blue
Background – Red to Light Pink

Discussion

All staining reagents should be stored at room temperature. Contamination will cause the solution to have a green cast and may affect the stain reaction. If contamination occurs, discard the solution. The iron 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 Working Iron Solution be discarded after use. The Nuclear Red Thiazin Stain Solution can be filtered and reused if desired. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

More intense iron staining is achieved with the room temperature procedure. 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

The ferric ion (Fe^{3+}), in the presence of an acidic solution and potassium ferrocyanide, will form an insoluble bright blue pigment (Prussian Blue). Only loosely bound ferric ions (such as in hemosiderin) are detected by this technique. Strongly bound iron (such as in hemoglobin) is not detected.

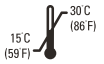
References

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. Carson. F.L. Histotechnology: A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, 1997.

Order Information

Product	Size	Qty.	REF
Iron Stain Kit	1 Kit	1	87006
Potassium Ferrocyanide Solution	250 mL	1	88013
Hydrochloric Acid Solution (20%)	250 mL	1	88014
Nuclear Red Thiazin Stain Solution	125 mL	1	88015

Anatomical Pathology



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