

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Periodic Acid-Schiff (PAS) Stain Instructions for Use

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

No special requirements; formalin fixation is adequate. Carnoy's Fixative may also be used.

Quality Control

A section of kidney is the most sensitive control. But a section of liver containing glycogen or a section of cervix with endo and ectocervix should be used in order to demonstrate glycogen.

Technical Procedure

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Place sections in Periodic Acid Solution for 5 minutes at room temperature.
3. Rinse sections in several changes of deionized water.
4. Stain sections in Schiff Reagent for 15 minutes to achieve desired contrast.
5. Rinse sections in lukewarm running tap water for 10 minutes.
6. Stain sections in Hematoxylin 1 for 1 minute to achieve desired contrast.
7. Rinse sections in deionized water for 30 seconds.
8. Place sections in Bluing Reagent for 1 minute.
9. Rinse sections in deionized water for 30 seconds.
10. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
11. 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 Periodic Acid Solution for 5 minutes at room temperature.
3. Rinse sections in deionized water for 30 seconds.
4. Place sections in plastic coplin jar containing Schiff Reagent.
5. Microwave sections on high for 30 seconds to maintain a temperature of 30°C.
6. Remove sections from microwave and agitate the solution to equilibrate the temperature. Allow sections to remain in warm solution for an additional 5 minutes.
7. Rinse sections in lukewarm running tap water for 10 minutes.
8. Stain sections in Hematoxylin 1 for 1 minute.
9. Rinse sections in deionized water for 30 seconds.
10. Place sections in Bluing Reagent for 1 minute.
11. Rinse sections in deionized water for 1 minute.
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

Carbohydrates, Glycogen, Basement Membranes, Fungus – Magenta

Nuclei – Blue

Background – Light Purple

Probable Mode of Action

Periodic acid oxidizes various tissue moieties to aldehydes. Schiff Reagent bonds to the induced tissue-aldehyde in its leuco (colorless) form. Water rinsing removes the sulfrurous acid, restoring the Schiff Reagent to its rose-colored form.

Discussion

It is strongly recommended to store Schiff Reagent tightly capped at 2-8°C in the laboratory refrigerator; this ensures longer stability. If stored improperly, Schiff Reagent may form a white precipitate. In most cases, small amounts of white precipitate will not impede on the stain performance. However, larger amounts of white precipitate can lead to a weak Schiff stain reaction. Contamination will cause the Schiff Reagent to discolor; it should be discarded. The PAS 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. The Periodic Acid Solution and Schiff Reagent should be discarded after use. Hematoxylin 1 can be filtered and reused if desired. 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. Bouin's and Zenker's Fixatives have shown adverse effects on staining results. 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.

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
Periodic Acid-Schiff (PAS) Kit	1 Kit	1	87007
Periodic Acid Solution (0.5%)	500ml	1	88016
Schiff Reagent	500ml	1	88017
Hematoxylin 1	500ml	1	88018

