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Thermo Scientific™ Richard-Allan Scientific™ Chromaview™ – Advanced Testing Alcian Blue pH 2.5 Periodic Acid Schiff Stain Instructions for Use

For in vitro diagnostic use.

For use as a kit in special staining techniques.

Technical Discussion

Microtomy
Cut paraffin sections at 4-6 microns.

Fixation
10% formalin, Thermo Scientific Pen-Fix or Carnoy's. Avoid fixatives containing gluteraldehyde to prevent non-specific background staining.

Quality Control
A section of kidney or mucin containing tissue (such as small intestine) should be used.

Technical Procedure

Standard Staining Protocol

1. Deparaffinize and hydrate to deionized water.
2. Place slides in Alcian Blue pH 2.5 Stain Solution for 30 minutes at room temperature.
3. Rinse sections in several changes of deionized water.
4. Place sections in Periodic Acid Solution for 5 minutes at room temperature.
5. Rinse sections in several changes of deionized water.
6. Stain sections in Schiff Reagent for 15 minutes.
7. Rinse sections in lukewarm running tap water for 10 minutes.
8. Rinse sections in several changes of deionized water.
9. Optional Nuclear Stain:
    a. Hematoxylin 7211 for 1 minute.
    b. Rinse sections in several changes of deionized water.
    c. Stain sections in Bluing Reagent for 1 minute.
    d. Rinse sections in running tap water for 1 minute.
10. Rinse sections in several changes of deionized water.
11. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
12. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Microwave Staining Protocol

1. Deparaffinize and hydrate to deionized water.
2. Place sections in a plastic coplin jar containing Alcian Blue pH 2.5 Stain Solution.
3. Microwave for 25 seconds at 80% power to achieve 60° C.
4. Incubate sections an additional 1 minute in heated Alcian Blue Solution.
5. Rinse sections in several changes of deionized water.
6. Place sections in Periodic Acid Solution for 5 minutes at room temperature.
7. Rinse sections in several changes of deionized water.
8. Place sections in a plastic coplin jar containing Schiff Reagent.
9. Microwave for 25 seconds at 80% power to achieve 60° C.
10. Remove sections from microwave and agitate the solution to equalize the temperature.
11. Allow sections to remain in warm solution for an additional 5 minutes.
12. Rinse in lukewarm running tap water for 10 minutes.
13. Optional Nuclear Stain:
    a. Hematoxylin 7211 for 1 minute.
    b. Rinse sections in several changes of deionized water.
    c. Stain sections in Bluing Reagent for 1 minute.
    d. Rinse sections in running tap water for 1 minute.
14. Rinse in deionized water.
15. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
16. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Acid Mucosubstances – Blue

Neutral Polysaccharides – Magenta

Other substances and mixtures of the above may appear purple. The color may range from blue purple to violet.

Nuclei – Blue if optional Hematoxylin 7211 counterstain is used

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. The Alcian Blue/PAS staining reagents are for "In Vitro" use only. Some of the reagents used in this kit are considered toxic. Refer to the Safety Data Sheet for Health and Safety Information. All reagents are stable and should not form precipitants under recommended storage parameters. For best results it is recommended that the Periodic Acid and Schiff Reagent be discarded after use. The Alcian Blue pH 2.5 Stain Solution may be filtered back and reused. Reagents used for the microwave technique should be discarded after use. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

Allow adequate time for complete deparaffinization and rehydration of sections before staining. Incomplete rehydration may result in poor staining with Alcian Blue pH 2.5 Stain Solution. 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. The Alcian Blue pH 2.5 Stain Solution may be used independently for detection of both carboxylated and sulfated acidic mucosubstances.

Probable Mode of Action

Alcian Blue reacts to compounds containing anionic groups such as acid mucosubstances and acidic mucins. At pH 2.5, both carboxylated and sulfated acidic mucosubstances are stained blue. Periodic acid is used to oxidize the tissue to form aldehyde groups that are demonstrated by the Schiff Reagent. Glycogen and neutral mucosubstances as well as basement membranes will be stained magenta by the Schiff Reagent. The tap water rinse develops and intensifies the magenta hues of the Schiff Reagent. An optional counterstain may be used to provide nuclear detail. Hematoxylin 7211 is recommended, however, Kernechtrot Nuclear Fast Red may be used if a red nuclear stain is desired.

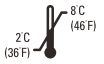
References

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: Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, 1997.

Order Information

Product	Size	Qty.	REF
Alcian Blue/PAS Kit	1 Kit	1	87023
Alcian Blue pH 2.5 Stain Solution	500 mL	1	88043
Periodic Acid Solution (0.5%)	500 mL	1	88016
Schiff Reagent	500 mL	1	88017
Hematoxylin 7211 (not included in kit)	1 pint	4/cs	7211

Anatomical Pathology



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