

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Alcian Blue pH 2.5 Stain Solution Instructions for Use

Technical Discussion

Microtomy

Cut paraffin sections at 4-6 microns.

Fixation

10% formalin, Thermo Scientific Pen-Fix or Carnoy's. Avoid fixatives containing glutaraldehyde 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

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. Optional Nuclear Stain: Kernechtrot Nuclear Fast Red Stain 1 minute.
5. Rinse sections in several changes of deionized water.
6. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
7. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Standard Staining Protocol Alcian Blue pH 2.5 PAS

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 (0.5%) 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. 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 Alcian Blue pH 2.5 PAS

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% 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. Rinse in deionized water for 1 minute.
14. 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.
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

Nuclei – Red if optional Kernechtrot Nuclear Fast Red counterstain is used

Discussion

The Alcian Blue pH 2.5 Stain Solution should be stored at room temperature and should not form precipitants under recommended storage parameters. The Alcian Blue pH 2.5 Stain Solution is for "In Vitro" use only. Refer to the Material Safety Data Sheet for health and safety information. The Alcian Blue pH 2.5 Stain Solution may be filtered back and reused; however, 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 level or times to achieve desired results.

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.

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
Alcian Blue pH 2.5 Stain Solution	500ml	1	88043
Periodic Acid Solution (0.5%)	500ml	1	88016
Schiff Reagent	500ml	1	88017
Kernechtrot Nuclear Fast Red Stain Solution	125ml	1	88049
Hematoxylin 7211	1pint	4/cs	7211

