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Thermo Scientific™ Richard-Allan Scientific™ Chromaview™ – Advanced Testing Acid Fast Bacillus Stain – Green Secondary Stain Instructions for Use

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

Technical Discussion

Microtomy
Cut sections at 4-5 microns.

Fixation
No special requirements; formalin fixation is adequate.

Quality Control
A section containing acid-fast microorganisms should be used.

Technical Procedure

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Stain sections in filtered Carbol Fuchsin Solution for 15 minutes.
3. Rinse sections in deionized water for 1 minute.
4. Dip sections in 2 changes of Differentiating Solution until tissue is pale pink.
5. Rinse sections in deionized water for 1 minute.
6. Counterstain sections in Fast Green Stain Solution 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 50ml of filtered Carbol Fuchsin Solution in a plastic coplin jar with lid applied loosely.
3. Microwave jar at 70% power for 40 seconds or as needed for final temperature of 80° C.
4. Remove jar from oven and add slides to jar. Let stand for 45 seconds with cover on.
5. Rinse sections in deionized water for 30 seconds.
6. Dip sections in 2 changes of Differentiating Solution until tissue is pale pink.
7. Rinse sections in deionized water for 1 minute.
8. Counterstain sections in Fast Green Stain Solution for 30 seconds.
9. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
10. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Acid Fast Organisms – Red
Background – Green

Discussion

All staining reagents should be stored at room temperature. The Acid Fast Bacillus 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 Differentiating Solution be discarded after use. The Carbol Fuchsin Solution and Fast Green Stain Solution can be filtered and reused. The stains should not be diluted and are ready-for-use. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

More intense acid fast bacteria staining is achieved with the room temperature procedure. Variations may occur in the results of the primary reaction due to the decolorization step and the individual's own staining technique. 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 capsule that surrounds the acid-fast bacterium has high-lipid content. This waxy, lipid-rich cell wall absorbs carbol-fuchsin dye and resists decolorization when acid-alcohols are used. However, bacteria that do not have a lipid-rich cell wall will easily decolorize. This is a useful technique to identify mycobacteria (Carson). Fast green solution provides a light counterstain for surrounding tissue elements.

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. Ramakrishnan, T., et. al,: Bacterial. Rev. 36: 1976.
5. Carson, F.L. Histotechnology: A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, 1997.

Order Information

Product	Size	Qty.	REF
Acid Fast Bacillus, Green Secondary Stain		1 Kit	1 87015
Carbol Fuchsin Solution	125 mL	1	88001
Fast Green Stain Solution	250 mL	1	88003
Differentiating Solution	500 mL	1	88117

Anatomical Pathology

15 C (59 F) – 30 C (86 F)

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