

RICHARD-ALLAN SCIENTIFIC

CHROMAVIEW®

Gram Stain
Tissue

American MasterTech
scientific laboratory supplies

1330 Thurman Street
Lodi, CA 95240

Phone: 800.860.4073
Fax: 209.368.4136

www.americanmastertech.com



Gram Stain

Tissue

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

No special requirements; formalin fixation is adequate.

Technical Procedure

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Stain sections in Crystal Violet Solution for 1 minute.
3. Rinse sections in running water for 1 minute.
4. Place sections in Gram's Iodine Solution for 5 minutes.
5. Rinse sections in running water for 30 seconds.
6. Differentiate sections in Decolorizing Solution.
7. Rinse sections in running water for 30 seconds.
8. Stain sections in Safranin O Stain Solution for 30 seconds.
9. Rinse sections in running water for 30 seconds.
10. Stain sections in Tartrazine Stain Solution for 20 seconds.
11. Dehydrate sections in two changes of anhydrous alcohol; 10 dips each.
12. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Elastic Fibers – Black

Gram Positive Organisms – Blue

Gram Negative Organisms – Red

Background – Yellow

Discussion

All staining reagents should be stored at room temperature. The Gram Stain 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. It is recommended that the Gram's Iodine Solution and Decolorizing Solution be discarded after use. The Crystal Violet Solution, Safranin O Stain Solution and the Tartrazine Stain Solution can be filtered and reused if desired. 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

If precipitation occurs in Crystal Violet Solution, place in warm oven (approximately 35°C) and stir. Gram's Iodine Solution may show a decrease in potency over time.

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.