

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Gram Stain – Tissue Instructions for Use

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

No special requirements; formalin fixation is adequate.

Quality Control

The use of a control slide with known Gram positive and known Gram negative organisms is recommended.

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 ensuring Tartrazine is retained on section.
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.

Probable Mode of Action

Bacteria can be classified into one of two families based upon the thickness of the peptidoglycan-containing cell wall. Gram positive bacteria have a thick peptidoglycan-containing cell wall. Gram negative bacteria have a thin peptidoglycan-containing cell wall. Both Gram positive and Gram negative bacteria stain with the dye lake created by the crystal violet stain and iodine mordant. However, during rinsing with Decolorizing Solution, the dye-lake is completely washed away from the thin, Gram negative bacteria. The cell wall will become counter-stained with the Safranin O Stain Solution. The short duration of the Decolorizing Solution rinse enables the dye lake to remain within the thicker, Gram positive cell wall. Take care when rinsing the slide with the Decolorizing Solution. Extended rinses with Decolorizing Solution can cause the crystal violet dye lake to wash out of the thick cell wall layer of Gram positive bacteria as well as the Gram negative bacteria. The tissue elements are counterstained with a yellow dye, tartrazine.

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.

Order Information

Product	Size	Qty.	REF
Gram Stain Kit - Tissue	1 Kit	1	87018
Tartrazine Stain Solution	500ml	1	88012
Crystal Violet Solution	500ml	1	88101
Gram's Iodine Solution	500ml	1	88102
Safranin O Stain Solution	500ml	1	88103
Decolorizing Solution	500ml	1	88104

