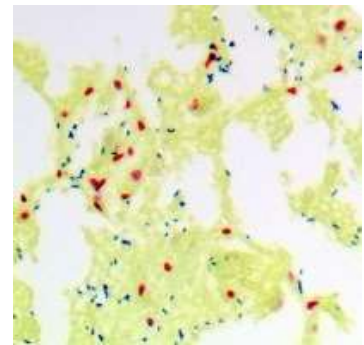


Gram Stain Kit (Modified Brown & Brenn)

Description

The Gram Stain Kit (Modified Brown & Brenn) is intended for the demonstration and differentiation of Gram-positive and Gram-negative bacteria.

Gram Positive Bacteria	Blue
Gram Negative Bacteria	Red
Other Tissue	Slightly Yellow – Pink
Nuclei	Red


Uses/Limitations

Not to be taken internally.
 Histological applications.
 Do not use if reagents become cloudy. Do not use past expiration date.
 Use caution when handling reagents. Non-Sterile.

Control Tissue

Any well fixed tissue section.
 Air dried smear.

Kit Contents

Product	Volume	Storage
Gentian Violet Solution	125 ml	18-25°C
Lugol's Iodine Solution	125 ml	18-25°C
Gram's Decolorizer Solution	125 ml	18-25°C
Safranin O Solution	125 ml	18-25°C
Picric Acid - Acetone Solution (0.1%)	125 ml	18-25°C

Precautions

Keep away from open flame.
 Avoid contact with skin and eyes. Harmful if swallowed.
 Follow all Federal, State, and local regulations regarding disposal.

**Procedure
(Standard)**

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Cover tissue section with Gentian Violet Solution and incubate for 2 minutes.
3. Rinse slide in distilled water to remove excess stain.
4. Cover tissue section with Lugol's Iodine Solution and incubate for 1 minute.
5. Rinse slide in running tap water to remove excess Iodine.
6. Dip 1 time in distilled water.
7. Apply Gram's Decolorizer dropwise or by repeated dips until color no longer bleeds off section.

8. Rinse slide quickly in distilled water (2-5 seconds).
9. Cover tissue section with Safranin O Solution and incubate for 4 minutes.
10. Rinse slide quickly in distilled water to remove excess stain.
11. Dip once in absolute alcohol and blot dry.
12. Cover tissue section with Picric Acid - Acetone Solution (0.1%) and incubate with agitation for 2-10 seconds.
13. Dehydrate slide very quickly in 3 changes of absolute alcohol.
14. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

References

1. Isenberg, H.H. Clinical Microbiology Procedures Handbook. American Society for Microbiology, 1992.
2. Sheehan, DC. Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 235.
3. Brown, J.H., Brenn, L. A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. Bulletin John Hopkins Hospital, 1931, Volume 48, pages 69-73.
4. Gram, C. Forstchr. Med., Volume 2, page 185, 1884.