



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: For In-Vitro Diagnostic use only.
Histological applications.
Do not use past expiration date.
Use caution when handling these reagents.

Control Tissue: Any well fixed paraffin embedded tissue section.

Availability/Contents:

<u>Item #</u>	<u>Kit Contents</u>	<u>Volume</u>	<u>Storage</u>
GVS030	Gentian Violet Solution	30 ml	18-25 °C
LIS030	Lugol's Iodine Solution	30 ml	18-25 °C
GDS030	Gram's Decolorizer Solution	30 ml x 2	18-25 °C
SOH030	Safranin O Solution	30 ml	18-25 °C
PAB030	Picric Acid - Acetone Solution (0.1%)	30 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.
Use in chemical fume hood whenever possible.

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 until color no longer bleeds off section.
8. Rinse slide quickly in distilled water.
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. Cover tissue section with Picric Acid - Acetone Solution (0.1%) and incubate with agitation for 5-10 seconds.
12. Dehydrate slide very quickly in 3 changes of absolute alcohol.
13. 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.