



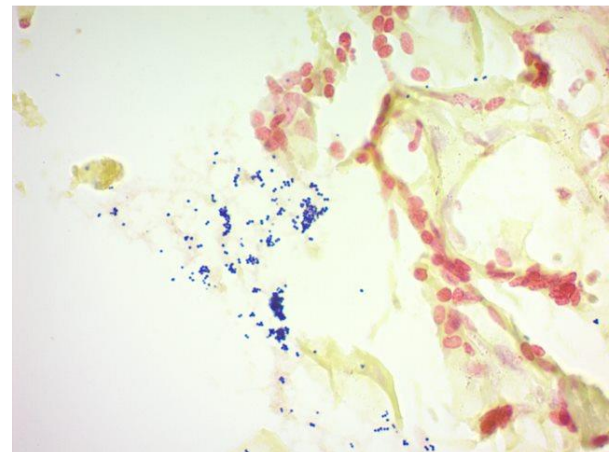
# Gram Stain Kit (Modified Brown & Brenn)

**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.  
For In-Vitro Diagnostic use only.  
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:**

<u>Item #</u>	<u>Product Name</u>	<u>Volume</u>	<u>Storage</u>
SSC1166	Gentian Violet Solution	500 ml	18-25°C
SSC1090	Lugol's Iodine Solution	500 ml	18-25°C
SSC1163	Gram's Decolorizer Solution	500 ml	18-25°C
SSC1188	Safranin O Solution	500 ml	18-25°C
SSC1106	Picric Acid - Acetone Solution (0.1%)	500 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. Festschr. Med., Volume 2, page 185, 1884.

Description:	Hazard Class	Packing Group	UN# Proper Shipping Name
Gentian Violet Solution	3	III	UN1993 <i>Flammable liquids, n.o.s. (Ethanol)</i>
Lugol's Iodine Solution	Not Regulated	Not Regulated	Not Regulated
Gram's Decolorizer	3	II	UN1993 <i>Flammable liquids, n.o.s. (Ethanol, Acetone)</i>
Safranin O Solution	Not Regulated	Not Regulated	Not Regulated
Picric Acid – Acetone Solution (0.1%)	3	II	UN1090 <i>Acetone</i>