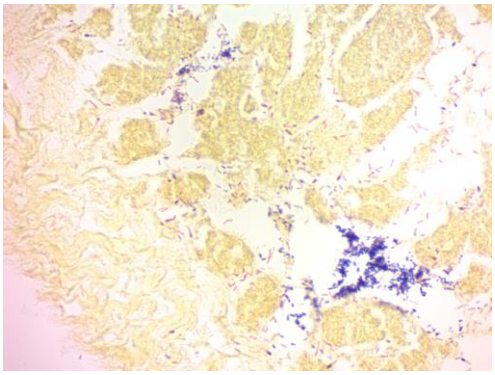
P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

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
Background:	Yellow
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.

Ordering Information and Current Pricing at www.scytek.com

Kit Contents:

Item #	Product Name	Volume	Storage
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
SOG030	Safranin O Solution (For Gram Staining)	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.

Storage: 18° C 25° C

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Procedure (Standard):

1. Deparaffinize sections if necessary and hydrate to deionized/distilled water.
2. Cover tissue section with Gentian Violet Solution and incubate for 2 minutes.
3. Rinse slide in deionized water to remove excess stain.
4. Cover tissue section with Lugol's Iodine Solution and incubate for 1 minute.
5. Rinse slide in deionized water to remove excess solution.
7. Carefully apply Gram's Decolorizer dropwise until color no longer bleeds off section. **Note:** *Application of this decolorizer for longer than 5 seconds may remove stain from gram positive bacteria.*
8. Rinse slide quickly in deionized water.
9. Cover tissue section with Safranin O Solution and incubate for 4 minutes.
10. Rinse slide in deionized water to remove excess stain.
11. Dip slide once in absolute alcohol and then remove excess alcohol from slide by blotting.
12. Carefully apply a few drops of Picric Acid - Acetone Solution (0.1%) while gently agitating for **2-10 seconds**, then immediately and briefly rinse slide in absolute alcohol. If tissue is still strongly red, repeat step 12 until it is mostly yellow – a tinge of red may remain due to presence of nuclei or large amounts of gram(-) bacteria.
13. Allow slide to air dry.
14. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

Note: Alcohol and Picric Acid-Acetone (steps 11-12) are required to remove red stain from background, but excess incubation with these solutions can also remove stain from bacteria.

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. Fosrtchr. Med., Volume 2, page 185, 1884.

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Bulk Reagent Ordering Information and Current Pricing at www.scytek.com

Description:	Catalog #	Volume
Gentian Violet Solution	GVS125	125 ml
	GVS500	500 ml
	GVS999	1000 ml
Lugol's Iodine Solution	LIS125	125 ml
	LIS500	500 ml
	LIS999	1000 ml
Gram's Decolorizer	GDS125	125 ml
	GDS500	500 ml
	GDS999	1000ml
Safranin O Solution (For Gram Staining)	SOG125	125 ml
	SOG500	500 ml
	SOG999	1000 ml
Picric Acid – Acetone Solution (0.1%)	PAB125	125 ml
	PAB500	500 ml
	PAB999	1000 ml

Storage: 18° C 25° C

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