

Instructions For Use

BBS-1-IFU

Rev. Date: Aug. 18, 2020

Revision: 3

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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.

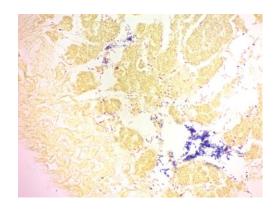
Do not use if reagents become cloudy. Do not use past expiration date. Use caution when handling reagents.

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
GVS125	Gentian Violet Solution	125 ml	18-25°C
LIS125	Lugol's Iodine Solution	125 ml	18-25°C
GDS125	Gram's Decolorizer Solution	125 ml	18-25°C
SOG125	Safranin O Solution (For Gram Staining)	125 ml	18-25°C
PAB125	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.

Storage: 18° C

ScyTek Laboratories, Inc. 205 South 600 West Logan, UT 84321 U.S.A.

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EC REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague, The Netherlands



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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.
- 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.
- 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.

Storage: 18° C 25° C

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

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

Storage: 18° C



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