ScyTek LABORATORIES INC.

Instructions For Use

GSK-IFU

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Gram Stain Kit

Description and Principle

The Gram Stain Kit is intended for the demonstration and differentiation of Gram-positive and Gram-negative bacteria due to differences in bacterial cell wall composition. Gram-positive and Gram-negative bacteria both have cell walls composed of peptidoglycan and lipoprotein, Gram-positive bacteria possess a much thicker peptidoglycan cell wall than Gram-negative bacteria.

Gentian violet and iodine form a dye complex that initially stains both gram-positive and gram-negative bacteria. The crystal violet-iodine complex is removed from gram-negative bacteria using Gram's Decolorizer solution while the dye is retained in the thick peptidoglycan cell wall of gram-positive bacteria. Carbol fuchsin is applied to counterstain gram-negative bacteria and tartrazine to stain background tissue.

Expected Results

Gram Positive Bacteria:	Blue
Gram Negative Bacteria:	Pink to Red
Other Tissue:	Yellow
Nuclei:	Red

Kit Contents

	Storage
1. Gentian Violet Solution	18-25°C
2. Lugol's Iodine Solution	18-25°C
3. Gram's Decolorizer Solution	18-25°C
4. Carbol Fuchsin Counterstain	18-25°C
5. Tartrazine Solution	18-25°C

Suggested Controls *(not provided)*

Tissue or cell smear containing both gram-positive and gram-negative organisms

Uses/Limitations

For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (18-25°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Procedure:

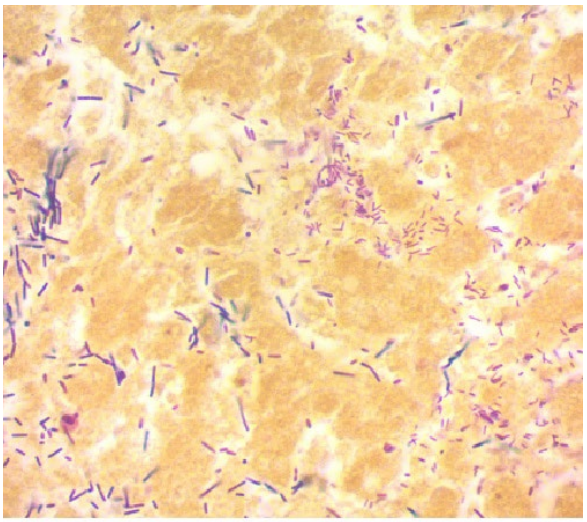
1. Deparaffinize sections if necessary and hydrate to distilled water.

2. Incubate slide in Gentian Violet Solution for 1 minute.

3. Rinse slide in distilled water to remove excess stain.

4. Incubate slide in Lugol's Iodine Solution for 1 minute.

5. Rinse slide in gently running tap water to remove excess Iodine.



Gram stain on Avian Liver demonstrating gram-positive and gram-negative bacteria viewed at 63x

6. Place slide in Gram's Decolorizer until color no longer bleeds off section. Note: Decolorization for longer than 5 seconds may remove stain from gram positive bacteria.

7. Rinse slide quickly in gently running tap water.

8. Incubate slide in Carbol Fuchsin Counterstain for 1-2 minutes.

9. Rinse slide quickly in gently running tap water to remove excess stain.

10. Incubate slide in Tartrazine Solution for 15 seconds.

11. Rinse slide 1 time in absolute alcohol.

12. Dehydrate slide quickly in 3 changes of absolute alcohol. Note: Dehydration in alcohols is necessary to remove background counterstain but should be done quickly to prevent excess decolorization of bacteria.

13. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

Other Notes: Gram positive bacteria that are dying, dead or being treated with antibiotics may stain variably (red).

References

1. Sheehan, DC., Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 235.
2. Su, R.J., Wang, P. Role of Gram stain in microbiological laboratories with limited resources. Reviews in Medical Microbiology. July 2011, Volume 22, Issue 3: pages 41-44. Doi: 10.1097/MRM.0b013e3283478a08.
3. Marira, J., Surekha, Y, Asangi, K.S., Suresh, B.S., Ramesh, S. Sputum Gram Stain Assessment in Relation to Sputum Culture for Respiratory Tract Infections in a Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. December 2011, Volume 5(8): pages 1699-1700.

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