



Instructions For Use KT 018-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

1. Gentian Violet Solution
2. Lugol's Iodine Solution
3. Gram's Decolorizer Solution
4. Carbol Fuchsin Counterstain
5. Tartrazine Solution

Storage

- 15-30°C
- 15-30°C
- 15-30°C
- 15-30°C
- 15-30°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 (15-30°C).

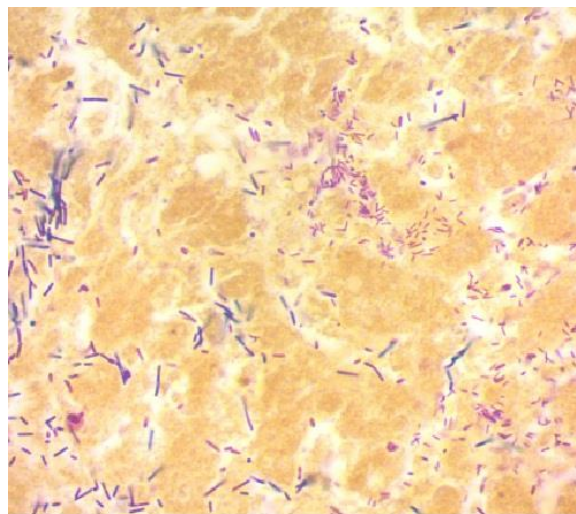
Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements. If there is any serious incident that has occurred in relation to the device, please contact the manufacturer: Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com. If required, please report to the Competent Authority of the Member State in which the user and/or patient is established.

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.



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