Diagnostic BioSystems

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Gram Stain Kit

Catalog Number: KT018

**This data sheet is applicable to all sizes (volume) of product. Actual volume is indicated on vial.

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The Gram Stain Kit is intended for the demonstration and differentiation of Gram positive and Gram-negative bacteria.

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

Control Tissue

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Reagents Provided

Kit Contents	Volume	Storage
Gentian Violet Solution	125 mL	15-30°C
Lugol's Iodine Solution	125 mL	15-30°C
Gram's Decolorizer Solution	125 mL	15-30°C
Carbol Fuchsin Counterstain	125 mL	15-30°C
Tartrazine Solution	125 mL	15-30°C

Storage and Handling

Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Cover tissue section with Gentian Violet Solution and incubate for 1 minute.
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. Place slide in Gram's Decolorizer until color no longer bleeds off section.
7. Rinse slide quickly in distilled water.
8. Cover tissue section with Carbol Fuchsin and incubate for 1-2 minutes.
9. Rinse slide quickly in distilled water to remove excess stain.
10. Apply Tartrazine Solution and incubate for 15 seconds.
11. Dehydrate slide quickly in 3 changes of absolute alcohol.
12. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.
7. Use in a chemical fume hood whenever possible.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

References

I. Sheehan, DC, Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 235.