Diagnostic BioSystems

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Acid Fast Bacteria (AFB) Stain Kit

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Acid Fast Bacteria (AFB) Stain Kit is intended for use in the histological visualization of Acid Fast Bacteria and Tubercle Bacilli. This kit is a rapid 15-minute procedure. The lipoid capsule of the acid-fast organism takes up Carbol Fuchsin and resists decolorization.

Acid Fast Organisms: Red
Background: Light Green

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
Carbol Fuchsin Solution	125 mL	15-30°C
Acid Alcohol Solution (0.5%)	500 mL	15-30°C
Light Green Solution	125 mL	15-30°C

Note
Carbol Fuchsin Solution should be filtered when a thick sheen develops on top of solution. To avoid possible contamination, gloves should be worn when performing this procedure. Do not use tap water prior to application of Carbol Fuchsin Solution as it is reported that Acid Fast Bacteria can be found in some systems. Use of distilled water is recommended whenever possible.

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 with distilled water.
2. Incubate slide in Carbol Fuchsin Solution for 15 minutes.
3. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
4. Decolorize in Acid Alcohol Solution (0.5%) until sections are a pale pink color.
5. Counterstain with Light Green Solution for 1-2 minutes.
6. Rinse several times in distilled water.
7. Dehydrate quickly through graded alcohols ending with 2 changes in absolute alcohol.
8. Clear, 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.

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. Sheenan, D.C., Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Battelle Press, Columbus, OH.
II. Carson, F.L., 1996, Histotechnology; A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, IL.

Diagnostic BioSystems
6616 Owens Drive
Pleasanton, CA 94588
Tel: (925) 484 3350
www. dbiosys.com

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Emergo Europe
Prinsessegracht 20
2514 AP, The Hague
The Netherlands
Tel: (31) (0) 70 345 8570