Diagnostic BioSystems

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Pneumocystis Stain Kit

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Pneumocystis Stain Kit is intended for use in the histological visualization of Pneumocystis carinii in cytology smears, and paraffin or frozen tissue sections.

Pneumocystis carinii: Violet / Purple
Connective Tissue: Blue / Green
Erythrocytes: Yellow
Mucin: Rose / Purple
Cartilage: Rose / Purple

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.

Recommended Positive Control

1. Pnuemocystis carinii

Reagents Provided

Kit Contents	Volume	Storage
Cresyl Echt Violet Solution (0.1%)	125 mL	2-8°C
Naphthol Yellow S Solution	125 mL	15-30°C
Staining Jar	60 mL	N/A

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.

Prepare Of Sulfation Reagent Immediately Before Use (Not Included in Kit):

1. Pour 15 ml of Glacial Acetic Acid into the Staining Jar provided with this kit.
2. Slowly add 5 ml of Sulfuric Acid to the Staining Jar.
3. Screw cap tightly on staining jar and invert several times to thoroughly mix acids.
4. Wait 5-10 minutes before proceeding with stain procedure to allow mixed acids to cool.
5. Use Staining Jar only for Sulfation procedure.
6. Wear protective clothing, gloves, and eyewear when mixing and handling this reagent. Make fresh for each use.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide in freshly mixed Sulfation Reagent for 10 minutes.
   Note: Agitate Staining Jar every few minutes to keep acids mixed.
3. Rinse in several changes of distilled water.
4. Incubate slide in Cresyl Echt Violet Solution (0.1%) for 10-15 minutes.
   Note: Agitate slide several times during incubation step.
5. Rinse quickly in distilled water.
6. Dip slide in Naphthol Yellow S Solution for 4 seconds.
   Note: Excessive Naphthol Yellow S Solution decolorizes Pneumocystis.
7. Rinse very quickly in distilled water.
8. Dehydrate very quickly in 2 changes of fresh Absolute Alcohol. Alternative Method: Dip slide twice in Absolute Alcohol and air-dry slide.
9. 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
This product is a single-use, non-sterile, in vitro diagnostic device.

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.

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

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EC REP

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

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