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Giemsa Stain Kit (May-Grunwald)

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The Giemsa Stain Kit (May-Grunwald) is intended for use in the visualization of cells present in hematopoietic tissues and certain microorganisms. This kit may be used on formalin fixed, paraffin-embedded or frozen sections.

Nuclei: Blue/Violet
Cytoplasm: Light Blue
Collagen: Pale Pink
Muscle Fibers: Pale Pink
Erythrocytes: Gray, Yellow or Pink
Rickettsia: Reddish-Purple
Helicobacter Pylori: Blue
Mast Cells: Dark Blue with Red Granules

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. Blood Film
2. Any well fixed tissue

Reagents Provided

Kit Contents	Volume	Storage
May-Grunwald Stock Solution	500 mL	15-30°C
Giemsa Stock Solution	500 mL	15-30°C
Phosphate Buffer Solution, pH 6.8	500 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.

Prepare the Following Solutions Immediately Before Use

1. Prepare Working May-Grunwald Solution by mixing 25ml of May-Grunwald Solution with 25ml of Phosphate Buffer Solution, pH 6.8.
2. Prepare Working Giemsa Solution by mixing 2.5ml of Giemsa Stock Solution with 50ml of Phosphate Buffer Solution, pH 6.8.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide in staining tray and flood with Working May-Grunwald Solution for 6 minutes. Note: Agitate slide occasionally to insure proper staining.
3. Flood slide with Phosphate Buffer Solution, pH 6.8 until no stain runs off.
4. Flood slide with Working Giemsa Solution for 13 minutes. Note: Agitate slide occasionally to insure proper staining.
5. Flood slide with Phosphate Buffer Solution, pH 6.8 until no stain runs off.
6. Allow slide to remain in Phosphate Buffer Solution, pH 6.8 for an additional 3 minutes.
7. Dip slide quickly in distilled water and air dry at room temperature.
8. Dip slide in Xylene or Xylene Substitute.
9. Mount in synthetic resin.

Procedure for Mast Cells

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide in staining tray and flood with Working May-Grunwald Solution for 6 minutes. Note: Agitate slide occasionally to insure proper staining.
3. Flood slide with Phosphate Buffer Solution, pH 6.8 until no stain runs off.
4. Flood slide with Working Giemsa Solution for 13 minutes. Note: Agitate slide occasionally to insure proper staining.
5. Flood slide with Phosphate Buffer Solution, pH 6.8 until no stain runs off.
6. Differentiate by dipping slide in Acetic Acid Solution (0.25%) until background is desired intensity.
7. Dip slide 20 times in Phosphate Buffer Solution, pH 6.8.
8. Dip slide quickly in distilled water and air dry at room temperature.
9. Dip slide in Xylene or Xylene Substitute.
10. 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.

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Precautions

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

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, D., Hrapchak, B., Theory and Practice of Histotechnology: 2nd Edition, 1980, pages 155-156.
II. Clark, G., et al. Staining Procedures. 4th Edition. Williams & Wilkins, 1981, pages 304-305.
III. A.F.I.P. Laboratory Methods in Histotechnology; 1992, pages 111.
IV. Laboratory Medicine: Vol. 25, No. 6, June 1994, page 389.

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