Giemsa Solution

Polychromatic solution of eosin, Methylene Blue and azure dyes
Used for staining in hematology, cytology and staining sections of hematopoietic organs in histopathology

REF 70840-1000: 1 lt,
70840-5000: 5 lt.

24 months, storage at RT

MenidiMedica
Biotechnology Applications

Introduction

Polychromatic Romanowsky dyes are a standard in hematology of blood smears and bone marrow. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Jenner and others) contain different ratios of methylene bluing reagent used as the cation component (and the reagent-related thiazine dyes, such as azure B) and eosin Y as the anion component. Cation and anion components interaction creates a well known Romanowsky effect that cannot be achieved if each component is being used individually. Purple color indicates the effect's presence. Staining intensity depends on the azure B content, as well as azure B to eosin Y ratio, while a few other factors affect the result of staining: working solution pH value and buffer solution, fixation method and dye exposure time. MenidiMedica Biotech Giemsa solution is used for differentiation of nuclear and/or cytoplasmatic morphology of lymphocytes, monocytes, granulocytes (neutrophils, eosinophils, basophils), thrombocytes and erythrocytes. There are various methods of using the Giemsa solution, and the so-called Pappenheim method is one of the most commonly used ones. The method is esentially the May-Gruenwald Giemsa method combined with the May-Gruenwald solution that stains cytological material (peripheral blood smears, cytodiagnostic puncture aspirates, diarrhea or secretion cells) or hematopoietic organs' sections. Along with the Pappenheim method, the Giemsa solution is commonly used for chromosomatic aberations detection in cytogenetics.

Product description

Solution of eosin, methylene bluing reagent and azure dyes in methanol and glycerol with added stabilizer.

Other sections and reagents that may be used in staining:

- Fixatives such as MenidiMedica Biotech neutral buffered formaldehyde solutions: Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as MenidiMedica Biotech alcohol solutions: Histanol 100
- Clearing agents, such as xylene or a substitute, such as agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as granulated paraffin
- High-quality glass slides for use in histopathology and cytology
- Fixatives
- MenidiMedica Biotech Immersion oil
- MenidiMedica Biotech Buffer solutions, pH 6.8 or 7.2

Working Giemsa solution for standard staining method

Add 10mL of the Giemsa solution to 190 ml of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filtrate if necessary.

Working Giemsa solution for rapid method

Add 33 mL of the Giemsa solution to 66 ml of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filtrate if necessary.

Working Giemsa solution for perioperative staining method

Add 10mL of the Giemsa solution to 50 ml of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filtrate if necessary.

A1) Blood smear staining procedure using Giemsa solution (standard method)

- Let the smear air dry
- Fix previously dried blood smears by immersing them in methanol - 5 min
- Immerse the fixed section into the working Giemsa solution - 15-20 minutes
- Rinse the smear in the pH 6.8 buffer solution - two exchanges - 2 exchanges, 1 minute each
- Air dry the slide

A2) Blood smear staining procedure using Giemsa solution (rapid method)

- Let the smear air dry
- Fix previously dried blood smears by immersing them in methanol - 1-3 min
- Immerse the fixed section into the working Giemsa solution- 3 minutes
- Rinse the smear in the pH 6.8 buffer solution - two exchanges - 2 exchanges, 1 minute each
- Air dry the slide

A3) Blood smear staining procedure using May-Gruenwald Giemsa (Pappenheim) standard method

- Let the smear air dry
- Apply May-Grunwald solution to the dried smear - 3-5 minutes
- Rinse the smear in pH 6.8 buffer solution.
- Apply working Giemsa solution to the smear - 15-20 minutes
- Rinse the smear in pH 6.8 buffer solution.

Note: If necessary, apply a smaller volume of the buffer solution on the slide in order to thoroughly remove the excessive dye and to make the stained structures clearly visible. Rinse the solution after 10-30 seconds.

- Air dry the slide

A4) Blood smear staining procedure using May-Gruenwald Giemsa (Pappenheim) perioperative method

- Let the smear air dry
- Apply May-Grunwald solution to the dried smear - 1-2 minutes
- Rinse the smear in pH 6.8 buffer solution.
- Apply working Giemsa solution to the smear - 5 min
- Rinse the smear in pH 6.8 buffer solution.

Note: If necessary, apply a smaller volume of the buffer solution on the slide in order to thoroughly remove the excessive dye and to make the stained structures clearly visible. Rinse the solution after 10-30 seconds.

- Air dry the preparation

Result (pH 6.8)

Nuclei - purple to violet
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light violet
Eosinophil granule - red
Basophil granule - dark violet to black
Thrombocytes - violet
Erythrocytes - reddish

Preparing the histological slides and solutions for the Giemsa solution staining (bone marrow biopsy, ilium biopsy)

- Fixate the sample (Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions 70, 80, 95, 100 (Histanol 100).
- Decalcify the sample by immersing it into a mild decalcifying agent (OsteoCal Mild Blue). Keep it immersed for 6 hours.
- Cut the sample carefully into small slices (5-20 µm). If necessary, treat it again with a decalcifying agent (OsteoCal Mild Blue) for 20 min.
- Clear the sample with intermedium; in xylene or in a xylene substitute.
- Infiltrate and embed the sample in paraffin.
- Cut the paraffin block to 4-6 µm slices and place them on a glass slide.

B) Histological slides staining procedure using Giemsa solution

- Deparaffinize the section using xylene or a xylene substitute, then rehydrate the section through series of descending alcohol solutions (Histanol 100), 95, 80, 70.
- Rinse the section with distilled water - 10 seconds
- Stain the section using Giemsa solution until it is optimally stained - 10-15 min

Note: Use undiluted Giemsa solution instead of the working solution in this step

- Differentiate the section using 0.1% acetic acid - 10 seconds
- Rinse the section with distilled water - 10 seconds
- Dehydrate the section through three exchanges of isopropyl alcohol - 3 exchanges, 10 seconds each
- Clear the section through two exchanges of xylene or a xylene substitute - 2 exchanges, 2 minutes each

Immediately after clearing apply an appropriate mount medium for covering/mounting on the section. If xylene was used, use one of mounting xylene-based media. If xylene substitute was used, use the appropriate covering agent. Cover the section with a cover glass.

Results

Nuclei - blue
Collagen, osteoid - light blue
Eosinophil granules - red
Acidophilic mucopolysaccharide, mastocytes, cartilage matrix - red-purple
Acidophilic substances - orange-red

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Reagents used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in MenidiMedica Biotech material safety data sheet.

Storing, stability and expiry date

Keep the Giemsa solution in a tightly closed original package at temperature between +15°C and +25°C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Beck, R.C. (1938): Laboratory Manual of Heamtological Technique, Philadelphia, W.B. Saunders & Co.
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3. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, Zentralb f Bakt; 89, pp 99-106.
4. International Commitee for Standardization in Haematology (1984): ICSH reference method for staining of blood and bone marrow films by azure B and eosin Y (Romanowsky stain), British Journal of Haematology, 57, p 707-710.
5. May, R. et Grünwald L. (1909): Über die Farbung von Feutchpraparaten mit meiner Azur-Eosine methode, Deutsche med Xschr, 35, pp 1751-1752.

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