

GIEMSA SOLUTION

IVD In vitro diagnostic medical device



Polychromatic eosin, methylene blue and azure dyes solution

Used for staining in hematology, cytology and staining sections of hematopoietic organs in histopathology

INSTRUCTIONS FOR USE

REF Product code: GM-OT-100 (100mL) GM-OT-110 (10x100mL) GM-OT-500 (500mL) GM-OT-1L (1000mL) GM-OT-2.5L (2500 mL)

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 blue 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, fixation method and dye exposure time. BioGnost's 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 essentially 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 chromosomal aberrations detection in cytogenetics.

Product description

- **GIEMSA SOLUTION** - solution of eosin, methylene blue and azure dyes in methanol and glycerol with added stabilizers.

Other products and reagents that may be used in staining:

- Fixative such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Polychromatic Romanowsky reagents, such as BioGnost's May-Gruenwald solution
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, Histanol 100 and Histanol IP
- Clearing agents, such as BioClear xylene or a substitute, for instance limonene-based BioNene or aliphatic hydrocarbon-based BioClear New agent
- Covering and mounting media such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam, or MountQuick Tube
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro, BioWax Plus 56/58
- Glass slides used in hematology, such as VitroGnost STANDARD GRADE or high quality glass slides used in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 types of VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- Immersion oils such as BioGnost's Immersion oil, Cedarwood oil, Immersion oil types 37, A, B, FF and NVH
- BioGnost's Buffer tablets, pH 6.8 or 7.2
- Fixative and differentiation agent, such as BioGnost's Histanol and Acetic acid for histology
- Decalcification agent used to treat calcified tissues in histology, such as BioGnost's OsteoSens

Preparation of solutions

Buffer solution, pH 6.8

- Dissolve 1 pH 6.8 buffer tablet in 1 liter of distilled water while stirring.

Note: During the staining process it is possible to use pH 7.2 buffer solution or a combination of pH 6.8 and 7.2 buffer solutions, and the process's results can differentiate in shift toward red or blue on the color spectrum.

Working Giemsa solution for standard staining method

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

Working Giemsa solution for perioperative staining method

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

0.1% acetic solution water solution

- Add 0.1mL of BioGnost's histology acetic acid to 99.9mL of distilled/demineralized water.

A1) Blood smear staining procedure using the Giemsa solution

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Fixate the previously dried blood smears by immersing them for 5 min in methanol (Histanol M).
- Immerse the fixated smear in the working Giemsa solution for 15-20 min.
- Rinse the smear twice in the pH 6.8 buffer solution during 1 min time.
- Dry the section.

Result (pH 6.8)

Nucleus - red to purple
 Lymphocyte plasma - blue
 Monocyte plasma - grey-blue
 Neutrophil granule - light purple
 Eosinophil granule - red to grey-blue
 Basophil granule - dark purple
 Thrombocytes - purple
 Erythrocytes - reddish
 Blood parasites - red nuclei

A2) Blood smear staining procedure using the May-Grünwald Giemsa (Pappenheim) standard method

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Let the smear dry.
- Apply the May-Grünwald solution to the dried smear and wait for 3-5 min.
- Rinse the smear shortly in pH 6.8 buffer solution.
- Apply the Giemsa solution to the dried smear and wait for 15-20 min.
- Rinse the smear shortly in pH 6.8 buffer solution.

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

- Dry the section.

A3) Blood smear staining procedure using the May-Grünwald Giemsa (Pappenheim) perioperative method

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Let the smear dry.
- Apply the May-Gruenwald solution to the dried smear and let it be active for 1-2 mins.
- Rinse the smear shortly in pH 6.8 buffer solution.
- Apply the Giemsa solution to the dried smear and let it be active for 5 min.
- Rinse the smear shortly in pH 6.8 buffer solution.

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

- Dry the section.

Result (pH 6.8)

Nucleus - purple

Lymphocyte plasma - blue

Monocyte plasma - grey-blue

Neutrophil granule - light purple

Eosinophil granule - red to dark purple

Basophil granule - dark purple to black

Thrombocytes - purple

Erythrocytes - reddish

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

- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Decalcify the sample by immersing it into a mild decalcifying agent (OsteoSens). Keep it immersed for 6 hours.
- Cut the sample carefully into small slices (5-20 μ m). If necessary, retreat it with a decalcifying agent (OsteoSens) for 20 min.
- Clear the sample with an intermedium; in xylene (BioClear) or in a xylene substitute (BioNene, BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro, BioWax Plus 56/58).
- Cut the paraffin block to 4-6 μ m slices and place them on a VitroGnost glass slide.

B) Histological sections staining procedure using the Giemsa solution

- Deparaffinize the section using xylene (BioClear) or a xylene substitute (BioNene or BioClear New), then rehydrate the section through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80 and Histanol 70).
- Rinse the section using distilled/demineralized water for 10 seconds.
- Stain the section with the Giemsa solution by immersing it for 10-15 min or until an optimal level of staining is achieved.

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

- Differentiate the section using the 0.1% acetic acid solution for 10 seconds.
- Rinse the section using distilled/demineralized water for 10 seconds.
- Dehydrate the section with three exchanges of isopropyl alcohol solution (Histanol IP), each exchange should last for 10 seconds.
- Clear the section through two xylene exchanges (BioClear) or a xylene substitute (BioNene, BioClear New), each should last for 5 minutes.
- Mount with appropriate medium. BioMount, BioMount High, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, Canada Balsam, or MountQuick Tube if BioClear xylene was used. If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New.
- Cover the section with a VitroGnost cover glass.

Result

Nucleus - 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 taken care of as a 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 BioGnost's material safety data sheet.

Storing, stability and expiry date

Keep Giemsa solution at temperature of 15 °C and 25 °C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Production date and expiry date are printed on the product's label.

References

1. Beck, R.C. (1938): *Laboratory Manual of Hematological Technique*, Philadelphia, W.B. Saunders & Co.
2. Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
3. Giemsa, G. (1922): Das Wesen der Giemsa-Färbung, *Zentralb f Bakt*; p89, pp99-106.
4. International Committee 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*, p57, pp707-710.
5. May, R. et Grünwald L. (1909): *Über die Färbung von Feuchtpreparaten mit meiner Azur-Eosine methode*, Deutsche med Xschr, p35, pp1751-1752.

GM-OT-X, V14-EN6, 23 December 2014, IŠP/VR

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				

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