

GIEMSA SOLUTION

IVD *In vitro* diagnostic medical device



Polychromatic solution of eosin, Methylene Blue and azure dyes

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

INSTRUCTIONS FOR USE

[REF] Catalogue number: GM-OT-100 (100mL) GM-OT-1010 (10x100 mL) GM-OT-500 (500 mL) GM-OT-1L (1000 mL) 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 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. BioGnost's Giemsa solution is used for differentiation of nuclear and/or cytoplasmic 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 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 BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- Fixatives, such as BioGnost's Histanol M
- BioGnost's Immersion oil
- BioGnost's Buffer tablets, pH 6.8 or 7.2

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 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.

0.1% acetic solution water solution

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

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

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

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

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

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

1.	Let the smear air dry	
2.	Apply May-Gruenwald solution to the dried smear	3-5 minutes
3.	Rinse the smear in pH 6.8 buffer solution.	
4.	Apply working Giemsa solution to the smear	15-20 minutes
5.	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.	
6.	Air dry the slide	

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

1.	Let the smear air dry	
2.	Apply May-Gruenwald solution to the dried smear	1-2 minutes
3.	Rinse the smear in pH 6.8 buffer solution.	
4.	Apply working Giemsa solution to the smear	5 min
5.	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.	
6.	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 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 μm). If necessary, treat it again with a decalcifying agent (OsteoSens) for 20 min.
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and embed the sample in paraffin (BioWax Plus, BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

B) Histological slides staining procedure using Giemsa solution

1.	Deparaffinize the section using xylene (BioClear) or a xylene substitute (BioClear New), then rehydrate the section through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80 and Histanol 70).	
2.	Rinse the section with distilled/demineralized water	10 seconds
3.	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	
4.	Differentiate the section using 0.1% solution of acetic acid	10 seconds
5.	Rinse the section with distilled/demineralized water	10 seconds
6.	Dehydrate the section through three exchanges of isopropyl alcohol (Histanol IP)	3 exchanges, 10 seconds each
7.	Clear the section through two exchanges of xylene (BioClear) or a xylene substitute (BioClear New)	2 exchanges, 2 minutes each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost 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 BioGnost's 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

- Beck, R.C. (1938): *Laboratory Manual of Hematological Technique*, Philadelphia, W.B. Saunders & Co.
- Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
- Giemsa, G. (1922): Das Wesen der Giemsa-Färbung, *Zentralbl f Bakt*; 89, pp 99-106.
- 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*, 57, p 707-710.
- May, R. et Grünwald L. (1909): *Über die Färbung von Feuchtpreparaten mit meiner Azur-Eosine methode*, Deutsche med Xschr, 35, pp 1751-1752.

GM-OT-X, V25-EN13, 22 May 2019, 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 in vitro diagnostic use only		Keep in dry place		Caution - fragile				

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