BIOGNOST®

MAY-GRUENWALD SOLUTION

IVD *In vitro* diagnostic medical device CE

Polychromatic solution of eosin, Methylene Blue and azure dyes
For use in hematology and cytology

INSTRUCTIONS FOR USE

REF Cat. number: MG-OT-100 (100 ml) MG-OT-110(10x100ml) MG-OT-500 (500 ml) MG-OT-1L (1000 ml) MG-OT-2.5 (2500 mL) MG-OT-20L (20 L)

Introduction

In hematology polychromatic Romanowsky dyes are a standard for blood smears and bone marrow staining. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Jenner) 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 May-Gruenwald solution is used for staining bone marrow and peripheral blood smear; for staining lymphocytes, monocytes, granulocytes (neutrophils, eosinophils and basophils), thrombocytes and erythrocytes. The May-Gruenwald solution is used in cytology to stain cytodiagnostic puncture aspirates, cells from diarrhea and secretion. One of the well known methods that use the May-Gruenwald solution is in combination with the Giemsa solution in the May-Gruenwald Giemsa, or Pappenheim method.

Product description

- **MAY-GRUENWALD SOLUTION** - Eosin and methylene bluing reagent solution in methanol with added stabilizers.

Other slides and reagents that may be used in staining:

- Polychromatic Romanowsky reagents, such as BioGnost's Giemsa solution
- 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 models of VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's Buffer tablets, pH 6.8 or 7.2
- Fixatives, such as BioGnost's Histanol M

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. The process's results can differentiate in shift toward red or blue on the color spectrum.

Diluted May-Gruenwald solution

- Combine 30 ml of May-Gruenwald solution with 150 ml of distilled or demineralized water and with 20 ml of buffer solution.

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 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 May-Gruenwald solution (using staining cell)

1.	Let the smear dry	
2.	Immerse the fixed blood smear in May-Gruenwald solution	3 min
3.	Immerse the fixed blood smear in diluted May-Gruenwald solution	6 min
4.	Rinse the smear in the pH 6.8 buffer solution - two exchanges	2 exchanges, 1 minute each
5.	Dry the preparation.	

A2) Blood smear staining procedure using May-Gruenwald solution (on the staining rack)

1.	Let the smear dry	
2.	Cover smear with 1 mL May-Gruenwald solution	3 min
3.	Add 1 mL buffer solution pH 6.8, mix and incubate	6 min
4.	Rinse the smear in the pH 6.8 buffer solution	
5.	Dry the preparation.	

Result (pH 6.8)

Nucleus - red-pink
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light purple
Eosinophil granule - red to red-brown
Basophil granule - dark purple to black
Thrombocytes - purple
Erythrocytes - reddish

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

1.	Let the smear 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.	Dry the slide	

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

1.	Let the smear 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.	Dry the preparation	

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

Note

Time periods of staining processes are not entirely standardized in clinical and laboratory practical experience. Time periods specified in the instruction approximately correspond to a longtime work practice with optimal results. 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 May-Gruenwald solution in a tightly closed original package at a temperature of +15 to +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.
2. Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
3. Garcia, L. S. (2001): *Diagnostic Medical Parasitology*, 4th ed., Washington, D.C., ASM Press.
4. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, *Zentralb f Bakt*; 89, p. 99-106.
5. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice,* 4th ed., Bloxham, Scion Publishing Ltd.
6. May, R. et Grünwald L. (1909): *Über die Farbung von Feutchpraparaten mit meiner Azur-Eosine methode,* Deutsche med Xschr, 35, p. 1751-1752.

MG-OT-X, V18-EN9, 5 May 2020, IŠP/VR

	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile						