BIOGNOST®

MAY-GRUENWALD SOLUTION

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Polychromatic solution of eosin, methylene blue, and azure dyes
For staining in hematology and cytology

INSTRUCTION FOR USE

BASIC UDI number	385889212HPC3010302HMCA	
EMDN code	W0103010302	
REF **Catalogue number**	**Volume**	**UDI-DI number**
MG-OT-100	100 mL	03858888822316
MG-OT-110	10x100 mL	03858888829001
MG-OT-500	500 mL	03858888822323
MG-OT-1L	1000 mL	03858888822330
MG-OT-2.5L	2500 mL	03858890001181

Intended use and testing principle

Polychromatic Romanowsky dyes are standardly used in hematology to stain blood smears and bone marrow. Various types of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Jenner) contain different ratios of methylene blue as a cationic component (and related thiazine dyes such as azure B) and eosin Y as an anionic component. The interaction of the cationic and anionic components creates the well-known Romanowsky effect, which cannot be achieved if each element is used separately, and is manifested by the formation of a purple color. The intensity of staining depends on the content of azure B and the ratio of azure B to eosin Y, while the staining result itself is influenced by several factors, including the pH value of the working solution and the buffer solution, the method of fixation, and the time of exposure to the dye. BioGnost's May-Gruenwald solution is used for staining bone marrow and peripheral blood smears; for staining lymphocytes, monocytes, granulocytes (neutrophils, eosinophils, and basophils), platelets, and erythrocytes. In cytology, cytodiagnostic puncture aspirates, cells from effusions and secretions are stained using May-Gruenwald solution. One of the most famous methods in which May-Gruenwald solution is used is in combination with Giemsa solution in the May-Gruenwald Giemsa, or Pappenheim method.

Product description

- **MAY-GRUENWALD SOLUTION** - A solution of eosin and methylene blue in methanol with the addition of a stabilizer.

Additional reagents and materials that can be used in the method

- Polychromatic Romanowsky reagents such as BioGnost Giemsa solution
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion media such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- BioGnost Buffer Tablets pH 6.8 or 7.2
- Fixation reagents such as BioGnost's Histanol M

Preparation of solutions

Buffer solution pH 6.8

- Disolve 1 buffer tablet pH 6.8 in 1 liter of distilled water with stirring.

Note: In the staining procedure, it is also possible to use a buffer solution with a pH value of 7.2 or a combination of buffer solutions with a pH value of 6.8 and 7.2. The results of the staining procedure may differ in a shift towards the red or blue color spectrum.

Diluted May-Gruenwald solution

- Mix 30 mL of May-Gruenwald solution with 150 mL of distilled/demineralized water and 20 mL of buffer solution.

Giemsa working solution for standard staining method

- Add 10 mL of Giemsa solution to 190 mL of pH 6.8 buffer solution, mix well, and let stand for 10 minutes. Filter if necessary.

Giemsa working solution for perioperative staining method

- Add 10 mL of Giemsa solution to 190 mL of pH 6.8 buffer solution, mix well, and let stand for 10 minutes. Filter if necessary.

NOTE

Make sure that the part of the slide with the sample is completely immersed in the appropriate solution or reagent in each step.

A1) Procedure for staining hematological smears and cytological samples with May-Gruenwald solution by immersion

1.	Air dry (fix) the blood smear or cytological sample on the slide	
2.	Immerse the fixed slide in May-Gruenwald solution	3 minutes
3.	Immerse the slide in May-Gruenwald solution	6 minutes
4.	Rinse the slide in buffer solution pH 6.8 through two changes	two 1-minute changes
5.	Air dry the slide	

A2) Procedure for staining hematological smears and cytological samples with May-Gruenwald solution on a rack

1.	Air dry (fix) the blood smear or cytological sample on the slide	
2.	Apply 1 mL of May-Gruenwald solution to the fixed slide	3 minutes
3.	Without pouring out the May-Gruenwald solution, add 1 mL of pH 6.8 buffer solution to the slide and mix gently. Let it sit	6 minutes
4.	Rinse the slide with buffer solution pH 6.8	
5.	Air dry the slide	

Result (pH 6.8)

Nuclei – pink purple color
Lymphocyte plasma – blue color
Monocyte plasma – gray-blue color
Neutrophil granule – light purple color
Eosinophilic granule – red to red-brown in color
Basophilic granule – dark purple to black color
Platelets – purple color
Erythrocytes – red color

A3) Procedure for staining blood smears using the May-Gruenwald Giemsa (Pappenheim) standard method

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

5.	Rinse the smear briefly in buffer solution pH 6.8.	
	Note: If necessary, leave a smaller volume of buffer solution on the slide to thoroughly remove stain residues and achieve a clear and sharp image of stained structures. Rinse off the solution after 10-30 seconds.	
6.	Air dry the slide.	

A4) Procedure for staining blood smears with the May-Gruenwald Giemsa (Pappenheim) perioperative method

1.	Air dry the blood smear.	
2.	Apply May-Gruenwald solution to the dried smear.	1-2 minutes
3.	Rinse the smear briefly in buffer solution pH 6.8.	
4.	Apply working Giemsa solution to the smear.	5 minutes
5.	Rinse the smear briefly in buffer solution pH 6.8.	
	Note: If necessary, leave a smaller volume of buffer solution on the slide to thoroughly remove stain residues and achieve a clear and sharp image of stained structures. Rinse off the solution after 10-30 seconds.	
6.	Air dry the slide.	

Result (pH 6.8)
Nuclei – purple color
Lymphocyte plasma – blue color
Monocyte plasma – gray-blue color
Neutrophil granule – light purple color
Eosinophilic granule – red to dark purple color
Basophilic granule – dark purple to black color
Platelets – purple color
Erythrocytes – reddish color

Limitations
This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics
Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, histological processing of samples and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control.
If a serious incident occurs during use or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection
Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

Storage, transport, stability, and shelf life
Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration date are printed on the product label.

Literature

1. Beck, RC (1938): Laboratory Manual of Hematological Technique, Philadelphia, WB Saunders & Co.
2. Dacie, J. et Lewis S. (1995): Practical hematology, 4th ed., London, Churchill Livingstone.
3. Garcia, LS (2001): Diagnostic Medical Parasitology, 4th ed., Washington, DC, ASM Press.
4. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, Zentralb f Bakt; 89, p. 99-106.
5. Kiernan, JA (2008): Histological and Histochemical Methods, Theory and Practice, 4th ed., Banbury: 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.

Warnings and precautions regarding the materials contained in the product:

H225 Highly flammable liquid and vapour.
H301+H311+H331 Toxic if swallowed, in contact with skin, or if inhaled.
H370 Causes damage to organs (eyes).

P21 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233 Keep container tightly closed.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310 IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P302+P352 IF ON SKIN: Wash with plenty of water.
P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P308+P311 IF exposed or concerned: Call a POISON CENTER or doctor/physician.

MG-IFU_EN12, 09.04.2025. LO/IŠP

	Manufacturer	LOT	Batch code		Consult Instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
12.	Extension of staining protocols A1 and A2 to cytological samples	09.04.2025.