BIOGNOST®

METHYLENE BLUE, C.I. 52015

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

For staining cells and microorganisms, component of Romanowsky hematology reagents

Methylene blue, Basic Blue 9

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30707PDYETD	
EMDN code	W01030707	
REF **Catalog number**	**Volume**	**UDI-DI**
MB-P-25	25 g	03858888820985
MB-P-100	100 g	03858888820992

Intended use and test principle

Histology, cytology, and other related scientific disciplines study the microscopic anatomy of tissues and cells. Proper staining is required to achieve good visualization of tissue and cellular structures. Methylene Blue is a basic (cationic) dye that binds to negatively charged (acidic) cellular structures, primarily nucleic acids (DNA and RNA), via electrostatic interactions. As a result, cell nuclei, bacteria, and other structures rich in nucleic acids take on a blue to dark blue color. This dye is used in several methods for staining cytological samples: for the preparation of Romanowsky polychromatic solutions for staining hematological samples, it is used as a counterstain in the staining of acid-fast bacteria, where its primary role is to stain almost all elements of bacterial cells, other cells, tissues, and background in a slightly alkaline medium, except for acid-fast bacteria which remain stained with the primary dye (most commonly Carbol Fuchsin reagent).

Product description

- **METHYLENE BLUE, C.I. 52015** - powder dye for the preparation of staining solutions for cytological (hematological) specimens and as a counterstain in microbiology

Example of using Methylene Blue powder dye as a component of May-Grünwald solution in the staining of hematological samples

Additional reagents and materials that can be used in the method

- Polychromatic Romanowsky reagents such as BioGnost's Giemsa solution
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- BioGnost's Buffer Tablets pH 6.8 or 7.2
- Fixatives such as BioGnost's Histanol M
- Eosin Y powder dye (cat. no. EOY-P-25, EOY-P-100, EOY-P-500)

Preparation of solutions

May-Gruenwald solution (100 mL)

- Dissolve 1 g of Methylene Blue powder dye in 100 mL of methanol. Add Eosin Y powder dye and stir until completely dissolved. Leave for several days (up to 7 days) at room temperature. Filter before use and store in a tightly closed amber bottle.

Buffer solution pH 6.8

- Dissolve 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-Grünwald solution with 150 mL of distilled/demineralized water and 20 mL of buffer solution.

Working Giemse solution for May-Gruenwald Giemsa (Pappenheim) standard staining method

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

NOTE

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

A1) Staining procedure for 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 min
3.	Immerse the slide in diluted May-Gruenwald solution	6 min
4.	Rinse the slide in buffer solution pH 6.8 through two changes	2 exchanges, 1 min each
5.	Air dry the slide	

A2) Staining procedure for 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 min
3.	Without pouring off the May-Gruenwald solution, add 1 mL of buffer solution pH 6.8 to the slide and mix gently. Allow to act	6 min
4.	Rinse the slide with buffer solution pH 6.8	
5.	Air dry the slide	

A3) Staining procedure for blood smears using May-Gruenwald Giemsa (Pappenheim) standard method

1.	Air dry the blood smear	
2.	Apply May-Gruenwald solution to the dried smear	3-5 min

3.	Briefly rinse the smear in buffer solution pH 6.8	
4.	Apply Giemsa working solution to the smear	15-20 min
5.	Briefly rinse the smear in buffer solution pH 6.8.	
	Note: If necessary, leave a small volume of buffer solution on the slide to thoroughly remove dye residues and achieve a clear and sharp image of the stained structures. Rinse off the solution after 10–30 seconds	
6.	Air dry the slide	

It is recommended to use immersion oil during microscopic analysis of the stained preparation at magnification greater than 40x.

Result (pH 6.8)
Nucleus – pink-purple
Lymphocyte cytoplasm – blue
Monocyte cytoplasm – gray-blue
Neutrophil granules – light purple
Eosinophil granules – red to red-brown
Basophil granules – dark purple to black
Platelets – purple
Erythrocytes – red

Limitations
This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the described staining solution preparation and staining procedure in these Instructions 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, solution preparation, staining, and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.
If a serious incident occurs during or as a result of the use of this product, please report it to the manufacturer and/or authorized representative and the 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, 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.

References

1. Conn, J. (1977): Biological Stains, 9th ed. Baltimore: Williams and Wilkins Co.
2. Carson, F. L., Hladik, C. (2009): Histotechnology: A Self-Instructional Text, 3rd ed., Chicago: ASCP Press
3. Lillie, R.O. (1969): Biological Stains, 8th ed., Baltimore: Williams & Wilkins Co.
4. Romanowsky, D.L. (1891): St. Petersburg Medizinische Wochenschrift 16: 297-302, 307-315

Warnings and precautions regarding the materials contained in the product:

H302	Harmful if swallowed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312	IF SWALLOWED: Call a POISON CENTER/doctor/ if you feel unwell.
P308+P313	IF exposed or concerned: Get medical advice/attention.

MB-P-IFU_ENV3, 21.04.2026. 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		

BioGnost Ltd.
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
3	Revised acc. to Regulation (EU) 2017/746 - IVDR	21.04.2026.