

# WRIGHT-GIEMSA SOLUTION



IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

## Polychromatic solution of Eosin Y, Methylene Blue and azure dyes For staining in hematology, cytology and cytogenetics

### INSTRUCTIONS FOR USE



<b>BASIC UDI number</b>	385889212HPC3010302HMCA				
<b>EMDN code</b>	W0103010302				
<b>REF</b> Catalog number	<b>Volume</b>	<b>UDI-DI number</b>	<b>REF</b> Catalog number	<b>Volume</b>	<b>UDI-DI number</b>
WRGM-OT-100	100 mL	03858890007541	WRGM-OT-1L	1000 mL	03858890009224
WRGM-OT-500	500 mL	03858890009217	WRGM-OT-2.5L	2500 mL	03858892121191

#### Intended use and test principle

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, Wright-Giemsa, 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 Wright-Giemsa solution is used for differentiating nuclear and/or cytoplasmic morphology of thrombocytes, erythrocytes, and lymphocytes in blood smear or bone marrow aspirates.

#### Product description

- **WRIGHT-GIEMSA SOLUTION** - Solution of Eosin Y, Methylene Blue and azure dyes in methanol with added stabilizer

#### Additional reagents and materials that can be used in this method

- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade
- BioGnost's Buffer tablets pH 6,8 or 7,2
- Fixatives such as BioGnost's Histanol M
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount DPX or BioMount New

#### Preparation of solutions

##### Buffer solution

- Dissolve 1 buffer tablet in 1 liter of distilled distilled/demineralized water while stirring. Filter after dissolving.

**Note:** During the staining process it is possible to use pH 6.8 or 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.

##### Working Wright-Giemsa solution for rapid staining (Wright-Giemsa to buffer solution ratio 1:3)

Working solution must be prepared in 1:3 ratio (for instance, add 30 ml of Wright-Giemsa solution to 60 ml of Buffer solution, pH 6.8 or 7.2 and stir well).

##### Working Wright-Giemsa solution for classic staining (Wright-Giemsa to buffer solution ratio 1:5)

Working solution must be prepared in 1:5 ratio (for instance, add 20 ml of Wright-Giemsa solution to 80 ml of Buffer solution, pH 6.8 or 7.2 and stir well).

#### NOTE

Make sure that the part of the slide with the sample is fully immersed into each corresponding solution or reagent at every step.

#### A1) Rapid staining procedure for peripheral blood smears using Wright-Giemsa solution

**Note: the staining procedure may be conducted in both horizontal and vertical positions**

1.	Air-dry the smear	
2.	Fix previously dried blood smears by immersing them in methanol (Histanol M)	1-3 min
3.	Air-dry the slide	
4.	Cover the slide with Wright-Giemsa working solution for rapid staining (1:3)	10 min
5.	Rinse the smear in pH 6.8 or pH 7.2 buffer solution (depending on which one was used for preparing working solution) - three exchanges*	3 exchanges, 30 sec each
6.	Air-dry the smear (the smear must be completely dry)	

\*In order to obtain permanent preparations, rinse them shortly in two Histanol 100 exchanges. Air-dry the smear (the smear must be completely dry) Immediately apply an appropriate BioMount medium for covering/mounting on the preparation after drying: BioMount DPX or BioMount New. Cover the preparation with VitroGnost cover glass.

#### A2) Classic staining procedure for peripheral blood smears using Wright-Giemsa solution

**Note: the staining procedure may be conducted in both horizontal and vertical positions**

1.	Air-dry the smear	
2.	Fix previously dried blood smears by immersing them in methanol (Histanol M)	1-3 min
3.	Air-dry the slide	
4.	Cover the slide with Wright-Giemsa working solution for standard staining (1:5)	20 min
5.	Rinse the smear in pH 6.8 or pH 7.2 buffer solution (depending on which one was used for preparing working solution) - three exchanges*	3 exchanges, 30 sec each
6.	Air-dry the smear (the smear must be completely dry)	

\*In order to obtain permanent preparations, rinse them shortly in two Histanol 100 exchanges. Air-dry the smear (the smear must be completely dry) Immediately apply an appropriate BioMount medium for covering/mounting on the preparation after drying: BioMount DPX or BioMount New. Cover the preparation with VitroGnost cover glass.

## Result

### Using pH 6,8 buffer solution

Nucleus – purple  
Lymphocyte cytoplasm – blue  
Monocyte cytoplasm – greyish or light blue  
Neutrophil granules – light red – purple  
Eosinophil granules – red – brown  
Basophil granules – dark blue - purple  
Thrombocyte granules – red – purple  
Erythrocytes – orange – red

### Using pH 7,2 buffer solution

Nucleus – purple  
Lymphocyte cytoplasm – blue  
Monocyte cytoplasm – greyish or light blue  
Neutrophil granules – light red – purple  
Eosinophil granules – red – brown  
Basophil granules – dark blue - purple  
Thrombocyte granules – red – purple  
Erythrocytes – greyish to orange  
Blood parasites – nuclei red – purple

## 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. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control. If a serious incident occurs during use of this product 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, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label 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 expiration date is printed on the product 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*, 4<sup>th</sup> ed., London, Churchill Livingstone.
3. Garcia, L. S. (2001): *Diagnostic Medical Parasitology*, 4<sup>th</sup> ed., Washington, D.C., ASM Press.
4. Giemsa, G. (1922): Das Wesen der Giemsa-Färbung, *Zentralbl f Bakt*; 89, str. 99-106.
5. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice*, 4<sup>th</sup> ed., Bloxham, Scion Publishing Ltd.
6. May, R. et Grünwald L. (1909): *Über die Färbung von Feuchtpreparaten mit meiner Azur-Eosine methode*, *Deutsche med Xschr*, 35, str. 1751-1752.

### Warnings and precautions regarding the materials contained in the product:

	H225 H301 + H311 + H331 H370	Highly flammable liquid and vapor. Toxic if swallowed, if on skin or if inhaled. Causes damage to organs (eyes).
	P210 P233 P280 P301 + P310 P302 + P352 P304 + P340 P308 + P311	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Keep container tightly closed. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: call immediately POISON CENTER/doctor. IF ON SKIN: wash with plenty of water IF INHALED: remove person to fresh air and keep comfortable for breathing. IF exposed or concerned: get medical advice/attention.

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 Manufacturer	 Batch code	 Consult instructions for use	 European conformity  Unique device identifier
 Date of manufacture	 Catalogue number	 Caution	
 Use-by date	 Temperature limit	 <i>In vitro</i> diagnostic medical device	

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Version	Description / reason for change	Date
5	Revised acc. to Regulation (EU) 2017/746 - IVDR	23.02.2026.