BIOGNOST®

# WRIGHT-GIEMSA SOLUTION

IVD In vitro diagnostic medical device CE

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

## INSTRUCTIONS FOR USE

REF Catalog number: WRGM-OT-100 (100 mL) WRGM-OT-500 (500 mL) WRGM-OT-1L (1000 mL) WRGM-OT-2.5L

**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, 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 cytoplasmatic morphology of thrombocytes, erythrocytes, and lymphocytes in blood smear or bone marrow aspirates.

**Product description**

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

**Other sections and reagents that may be used in staining:**

- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- 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 is used for hematology and cytology smears.**

**Buffer solution pH 7.2 is used for staining hematology smears expected to contain blood parasites.**

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

**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).

**A1) Staining procedure for peripheral blood smears using Wright-Giemsa solution**

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

| | | |
|---|---|---|
| **1.** | Dry the smear | |
| **2.** | Fix previously dried blood smears by immersing them in methanol (Histanol M) | 1-3 minutes |
| **3.** | Dry the preparation | |
| **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 seconds each |
| **6.** | Air-dry the smear (the smear must be completely dry) | |
| | *Note for obtaining permanent preparations*:<br>In order to obtain permanent preparations, rinse them shortly in two Histanol 100 exchanges.<br>Air-dry the smear (the smear must be completely dry)<br>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.** | Dry the smear | |
| **2.** | Fix previously dried blood smears by immersing them in methanol (Histanol M) | 1-3 minutes |
| **3.** | Dry the preparation | |
| **4.** | Cover the slide with **Wright-Giemsa working solution** for **classic** 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 | 3 exchanges, 30 seconds each |

| | | |
|---|---|---|
| | working solution) - three exchanges | |
| **6.** | Air-dry the smear (the smear must be completely dry) | |
| | *Note for obtaining permanent preparations*:<br>In order to obtain permanent preparations, rinse them shortly in two Histanol 100 exchanges.<br>Air-dry the smear (the smear must be completely dry)<br>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 (pH 6.8)**

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

**Result (pH 7.2)**

Nucleus - purple
Lymphocyte cytoplasm - blue
Monocyte cytoplasm - grey 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-red
Blood parasites - red-purple nuclei

**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 Wright-Glemsa 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**


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, p99-106.
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, pp1751-1752.



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| Refer to the supplied documentation | Storage temperature range | Number of tests in package | REF | Product code | CE | European Conformity | BIOGNOST Ltd.<br>Medjugorska 59<br>10040 Zagreb<br>CROATIA<br>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 | | | | | | |