BIOGNOST®

# GIEMSA SOLUTION

CE 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

### Used for staining in hematology, cytology and staining sections of hematopoietic organs in histopathology

**INSTRUCTIONS FOR USE**

| **BASIC UDI broj** | 385889212HPC3010302HMCA | | | | |
|---|---|---|---|---|---|
| **EMDN code** | W0103010302 | | | | |
| REF **Catalog number** | **Volume** | **UDI-DI number** | REF **Catalog number** | **Volume** | **UDI-DI number** |
| GM-OT-100 | 100 mL | 03858888822095 | GM-OT-1L | 1000 mL | 03858888822118 |
| GM-OT-110 | 500 mL | 03858888829018 | GM-OT-2.5L | 2500 mL | 03858888829919 |
| GM-OT-500 | 1000 mL | 03858888822101 | GM-OT-20L | 20000 mL | 03858890001723 |

**Intended use and test principle**

Polychromatic Romanowsky dyes are a standard in hematology of blood smears and bone marrow. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Jenner and others) 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 Giemsa solution is used for differentiation of nuclear and/or cytoplasmatic morphology of lymphocytes, monocytes, granulocytes (neutrophils, eosinophils, basophils), thrombocytes and erythrocytes. There are various methods of using the Giemsa solution, and the so-called Pappenheim method is one of the most commonly used ones. The method is esentially the May-Gruenwald Giemsa method combined with the May-Gruenwald solution that stains cytological material (peripheral blood smears, cytodiagnostic puncture aspirates, diarrhea or secretion cells) or hematopoietic organs' sections. Along with the Pappenheim method, the Giemsa solution is commonly used for chromosomatic aberations detection in cytogenetics.

**Product description**

- **GIEMSA SOLUTION** - solution of Eosin Y, Methylene Blue and azure dyes in methanol and glycerol with added stabilizer.

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

- Polychromatic Romanowsky reagents such as BioGnost's May-Gruenwald solution
- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- Decalcifying agent such as BioGnost's OsteoSensa
- 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
- BioGnost's Acetic acid for histology

**Preparation of solutions**

**Buffer solution pH 6,8**

- Dissolve 1 pH 6.8 buffer tablet in 1 liter of distilled/demineralized 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, and the process's results can differentiate in shift toward red or blue on the color spectrum.

**Working Giemse solution for 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.

**Working Giemsa solution for perioperative staining method**

- Add 10 mL of the Giemsa solution to 50 mL of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.

**Working Giemsa solution for rapid staining**

- Add 33 mL of the Giemsa solution to 66 mL of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.

**0.1% aqueous acetic solution**

- Add 0.1 mL of BioGnost's Acetic acid for histology to 99.9 mL of distilled/demineralized water.

**A1) Blood smear staining procedure using Giemsa solution**

| | | |
|---|---|---|
| **1.** | Let the smear air dry | |
| **2.** | Fix previously dried blood smears by immersing them in methanol (Histanol M) | 5 min |
| **3.** | Immerse the fixed section into the working Giemsa solution | 15-20 min |
| **4.** | Rinse the smear in the pH 6.8 buffer solution - two exchanges | 2 exchanges, 1 min each |
| **5.** | Air dry the slide | |

**A2) Blood smear staining procedure using Giemsa solution (rapid method)**

| | | |
|---|---|---|
| **1.** | Let the smear air dry | |
| **2.** | Fix previously dried blood smears by immersing them in methanol (Histanol M) | 1-3 min |
| **3.** | Immerse the fixed section into the working Giemsa solution | 3 min |
| **4.** | Rinse the smear in the pH 6.8 buffer solution - two exchanges | 2 exchanges, 1 min each |
| **5.** | Air dry the slide | |

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

| | | |
|---|---|---|
| **1.** | Let the smear air dry | |
| **2.** | Apply May-Gruenwald solution to the dried smear | 3-5 min |
| **3.** | Rinse the smear briefly in pH 6.8 buffer solution. | |
| **4.** | Apply working Giemsa solution to the smear | 15-20 min |
| **5.** | Rinse the smear briefly 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.** | Air dry the slide | |

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

| | | |
|---|---|---|
| **1.** | Let the smear air dry | |
| **2.** | Apply May-Gruenwald solution to the dried smear | 1-2 min |
| **3.** | Rinse the smear briefly in pH 6.8 buffer solution | |
| **4.** | Apply working Giemsa solution to the smear | 5 min |
| **5.** | Rinse the smear briefly 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.** | Air dry the slide | |

**Result (pH 6,8)**

Nuclei – purple to violet
Lymphocyte cytoplasm – plava boja (
Monocyte cytoplasm – greyish blue
Neutrophil granules – light violet
Eosinophil granules – light red
Basophil granules – dark purple to black
Thrombocytes – violet
Erythrocytes – reddish

**Preparing the histological slides and solutions for the Giemsa solution staining (bone marrow biopsy, ilium biopsy)**

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100)
- Decalcify the sample by immersing it into a mild decalcifying agent (OsteoSens). Keep it immersed for 6 hours
- Cut the sample carefully into small slices (5-20 $\mu$m). If necessary, treat it again with a decalcifying agent (OsteoSens) for 20 min
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New)
- Infiltrate and embed the sample in paraffin (BioWax Plus, BioWax 52/54, BioWax 56/58, BioWax Blue)
- Cut the paraffin block to 4-6 $\mu$m slices and place them on a VitroGnost glass slide.

**B) Histological slides staining procedure using Giemsa solution**

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|---|---|---|
| **1.** | Deparaffinize using xylene (BioClear) or xylene substitute (BioClear New), then rehydrate through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80 and Histanol 70). | |
| **2.** | Rinse with distilled/demineralized water | 10 sec |
| **3.** | Stain using Giemsa solution until it is optimally stained | 10-15 min |
| | Note: Use undiluted Giemsa solution instead of the working solution in this step | |
| **4.** | Differentiate using 0.1% solution of acetic acid | 10 sec |
| **5.** | Rinse with distilled/demineralized water | 10 sec |
| **6.** | Dehydrate through three exchanges of isopropyl alcohol (Histanol IP) | 3 exchanges, 10 sec each |
| **7.** | Clear through two exchanges of xylene (BioClear) or a xylene substitute (BioClear New) | 2 exchanges, 2 min each |

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). Cover the sample with a VitroGnost cover glass.

**Result**

Nuclei – blue
Collagen, osteoid – light blue
Eosinophil granules – red
Acidophilic mucopolysaccharide, mastocytes, cartilage matrix – red – purple
Acidophilic substances – orange – red

**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 Heamtological Technique,* Philadelphia, W.B. Saunders & Co.
2. Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
3. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, *Zentralb f Bakt*; 89, str. 99-106.
4. International Commitee for Standardization in Haematology (1984): ICSH reference method for staining of blood and bone marrow films by azure B and eosin Y (Romanowsky stain), *British Journal of Haematology,* 57, str. 707-710.
5. May, R. et Grünwald L. (1909): *Über die Farbung von Feutchpraparaten mit meiner Azur-Eosine methode,* Deutsche med Xschr, 35, str. 1751-1752.


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

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| H226 | Highly flammable liquid and vapor. |
| H301+H311+H331 | Toxic if swallowed, if on skin or if inhaled. |
| H370 | Causes damage to organs (eyes). |
| P210 | 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: call immediately 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: get medical advice/attention. |

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| | 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 |
|---|---|---|
| 14 | Revised acc. to Regulation (EU) 2017/746 - IVDR | 23.02.2026. |