BIOGNOST®

# MAY-GRUENWALD SOLUTION

IVD In vitro diagnostic medical device CE

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

## INSTRUCTIONS FOR USE

REF Product code: MG-OT-100 (100mL) MG-OT-110 (10x100mL) MG-OT-500 (500mL) MG-OT-1L (1000mL) MG-OT-2.5L (2500 mL)

**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, Jenner) contain different ratios of methylene blue (and related thiazine dyes, such as azure B) used as the cation component 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. Violet 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, fixation method and dye exposure time. BioGnost's May-Gruenwald solution is used for staining bone marrow and peripheral blood smear; for staining lymphocytes, monocytes, granulocytes (neutrophils, eosinophils and basophils), thrombocytes and erythrocytes. The May-Gruenwald solution is used in cytology to stain cytodiagnostic puncture aspirates, cells from diarrhea and secretion. One of the well known methods that uses the May-Gruenwald solution is in combination with the Giemsa solution in the May-Gruenwald Giemsa, or Pappenheim method.

**Product description**

- **MAY-GRUENWALD SOLUTION** - Eosin and methylene blue solution in methanol with added stabilizers.

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

- Fixative such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Polychromatic Romanowsky reagents, such as BioGnost's Giemsa solution
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, for instance limonene-based BioNene or aliphatic hydrocarbon-based BioClear New agent
- Covering and mounting media such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam, or MountQuick Tube
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro, BioWax Plus 56/58
- Glass slides used in hematology, such as VitroGnost STANDARD GRADE or high quality glass slides used in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 types of VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- Immersion oils such as BioGnost's Immersion oil, Cedarwood oil, Immersion oil types 37, A, B, FF and NVH
- BioGnost's Buffer tablets, pH 6.8 or 7.2
- Fixatives, such as BioGnost's Histanol M

**Preparation of solutions**

**Buffer solution, pH 6.8**

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

**Dissolved May-Gruenwald solution**

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

**Giemsa working solution**

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

**A1) Blood smear staining procedure using the May-Gruenwald method**

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Fixate the previously dried blood smears by immersing them for 1 min in methanol (Histanol M).
- Let the smear dry.
- Immerse the fixated dried smear in the May-Grünwald solution for 3-4 min.
- Immerse the fixated dried smear in dissolved May-Grünwald solution for 6 min.
- Rinse the smear twice in the pH 6.8 buffer solution during 1 min time.
- Dry the preparation.

**Result (pH 6.8)**

Nucleus - red-pink
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light purple
Eosinophil granule - red to red-brown
Basophil granule - dark purple to black
Thrombocytes - purple
Erythrocytes - reddish

**A2) Blood smear staining procedure using the May-Grünwald Giemsa (Pappenheim) method**

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Let the smear dry.
- Apply the May-Gruenwald solution to the dried smear and wait for 5 min.
- Rinse the smear in pH 6.8 buffer solution.
- Apply the Giemsa solution to the dried smear and wait for 25 min.
- Rinse the smear in pH 6.8 buffer solution.
  Note: If necessary, apply a smaller volume of the buffer solution on the preparation in order to thoroughly remove the excessive dye and to make the stained structures clearly visible. Rinse the solution after 10-30 seconds.
- Dry the preparation.

**Result (pH 6.8)**

Nucleus - purple
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light purple
Eosinophil granule - red to dark purple
Basophil granule - dark purple to black
Thrombocytes - purple
Erythrocytes - reddish

**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 taken care of as a 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**

May-Gruenwald should be stored at temperature between 15 °C and 25 °C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Production date 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, p 99-106
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, p 1751-1752



MG-OT-X, V14-EN5, 2 Feb 2015, IŠP/VR


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| | Refer to the supplied documentation | °C | Storage temperature range | Σ | 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 | | | | | | |