BIOGNOST®

# LEISHMAN'S SOLUTION

IVD In vitro diagnostic medical device CE

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

## INSTRUCTIONS FOR USE

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

**Introduction**

Romanowsky polychromatic dyes are standardly used in hematology and cytology. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, 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. Known Romanowsky effect is created by mutual interaction between cationic and anionic components, and it is being demonstrated by purple color. Staining intensity depends on the azure content, as well as azure 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.

**Product description**

**LEISHMAN'S SOLUTION** - Solution of eosin, Methylene Blue and azure in methanol.

**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**

Filter undiluted Leishman's solution before use.

**Buffer solutions**

Buffer solution pH 6.8 (for hematology and cytology smears).
Buffer solution pH 7.2 (for staining hematology smears expected to contain blood parasites).

- Dissolve 1 buffer tablet in 1 liter of distilled 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.

**Leishman's working solution for vertical staining**

- Combine 30mL of Leishman's solution with 150 ml of distilled or demineralized water and with 20 ml of pH 6.8 or pH 7.2 buffer solution. Let it set for 10 min. Filter before use.

**Working Leishman's solution for staining in automatic stainer**

- Combine 50mL of Leishman's solution with 220 ml of distilled or demineralized water and with 30 ml of pH 6.8 or pH 7.2 buffer solution. Let it set for 10 min. Filter before use.

**A1) Procedure of horizontal smear staining (on a staining rack)**

| | | |
|---|---|---|
| **1.** | Dry the preparation | |
| **2.** | Place the section in the horizontal position and cover it with 1 ml of **undiluted Leishman's solution** | 1 min |
| **3.** | Add 2 mL of **Buffer solution** pH 6.8 or pH 7.2 , gently stir and let it react | 5 min |
| **4.** | Rinse with **Buffer solution,** pH 6.8 or pH 7.2 through two exchanges | 2 exchanges, 1 minute each |
| **5.** | Dry the preparation | |

**A2) Procedure of vertical smear staining (in a staining jar)**

| | | |
|---|---|---|
| **1.** | Dry the preparation | |
| **2.** | Immerse the section into **non-diluted Leishman's solution** | 3 min |
| **3.** | Immerse the section into **Leishman's working solution for vertical staining** | 6 min |
| **4.** | Rinse with **Buffer solution,** pH 6.8 or pH 7.2 through two exchanges | 2 exchanges, 1 minute each |
| **5.** | Dry the slide | |

**A3) Procedure of staining smear in automatic stainer**

| | | |
|---|---|---|
| **1.** | Dry the preparation | |
| **2.** | Immerse the section into **non-diluted Leishman's solution** | 3 min |
| **3.** | Immerse the section into **Leishman's working solution for staining in automatic stainer** | 6 min |
| **4.** | Rinse with **Buffer solution,** pH 6.8 or pH 7.2 | 1 min |
| **5.** | Rinse in tap water | 2 min |
| **6.** | Dry the preparation | |

| | Result (pH 6.8) | Result (pH 7.2) |
|---|---|---|
| Nucleus | red-purple | purple |
| Lymphocyte cytoplasm | blue | light blue |
| Monocyte cytoplasm | grey-blue | blue-grey |
| Neutrophil granules | bright purple | light red-purple to pink |
| Eosinophil granules | dark red | red-pink to brown-red |
| Basophil granules | dark purple | dark blue-purple to black |
| Thrombocyte granules | purple | reddish-purple |
| Erythrocytes | reddish | reddish-grey |
| 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 Leishman's 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, p 99-106.
5. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice,* 4th ed., Bloxham, Scion Publishing Ltd.


LE-OT-X, V10-EN8, 21 May 2019, IŠP/VR

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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 | | | | | | |