BIOGNOST®

LEISHMAN'S 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

For staining in hematology, cytology and cytogenetics

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC3010302HMCA	
EMDN code	W0103010302	
REF **Catalog number**	**Volume**	**UDI-DI number**
LE-OT-100	100 mL	03858888822262
LE-OT-500	500 mL	03858888822279
LE-OT-1L	1000 mL	03858888822286

Intended use and test principle

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 Y, methylene blue and azure in methanol

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

Preparation of solutions

Filter undiluted Leishman's solution before use.

Leishman's working solution for vertical staining

Combine 30 mL of Leishman's solution with 150 ml of distilled/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

Leishman's working solution for staining in automatic stainer

Combine 50 mL of Leishman's solution with 220 ml of distilled/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

Buffer solution pH 6,8

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

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.

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) Procedure for horizontal staining of hematological smears and cytological samples (on a staining rack)

1.	Dry (fix) the blood smear or cytology sample on the slide	
2.	Place the sample 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 min each
5.	Dry the sample	

A2) Procedure for vertical staining of hematological smears and cytological samples (in a staining jar)

1.	Dry (fix) the blood smear or cytology sample on the slide	
2.	Immerse the sample into **undiluted Leishman's solution**	3 min
3.	Immerse the sample 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 min each
5.	Dry the sample	

A3) Procedure of staining hematological smears and cytological samples in automatic stainer

1.	Dry (fix) the blood smear or cytology sample on the slide	
2.	Immerse the sample into **undiluted Leishman's solution**	3 min
3.	Immerse the sample 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 under tap water	2 min
6.	Dry the sample	

It is recommended to use immersion oil during microscopic analysis with magnification over 40x.

Result

	Using pH 6,8 buffer solution	Using pH 7,2 buffer solution
Nucleus	Red – purple	Purple
Lymphocyte cytoplasm	Blue	Light blue
Monocyte cytoplasm	Blue - grey	Blue – grey
Neutrophil granules	Light purple (u staroj piše "bright")	Light red – purple to pink
Eosinophil granules	Dark red	Red – pink to brown
Basophil granules	Dark purple	Dark purple
Thrombocytes	Purple	Reddish purple
Erythrocytes	Reddish	Reddish – grey
Blood parasites	/	Red – purple nuclei

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.

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. 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, str. 99-106.
5. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice,* 4th ed., Bloxham, Scion Publishing Ltd.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapor.
H301	Toxic if swallowed.
H311	Toxic if on skin.
H331	Toxic 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		

BioGnost Ltd.
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

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