BIOGNOST®

LEISHMAN STAIN powder dye

IVD In vitro diagnostic medical device CE

Leishman stain, Eosin-polychrome Methylene Blue
For staining blood smears

INSTRUCTIONS FOR USE

REF Product code: LS-P-25 (25 g)

Introduction

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Leishman powder dye is intended for microscopy staining using different methods. It is a typical purple dye for cell nuclei because of its molecular interactions between Eosin Y and Azure B-DNA complex. Staining intensity depends on the amount of Azure B and Azure B/Eosin Y ratio. Leishman stain is classified as a Romanowsky stain. It is used for differentiating leukocytes because staining results in different hues ranging from red to blue.

Product description

- **LEISHMAN STAIN** - Powder dye for creating dye solution for staining blood smears.

Other slides and reagents that may be used in staining:

- 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 models of VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's Buffer tablets, pH 6.8 or 7.2
- Fixatives, such as BioGnost's Histanol M

Preparation of solutions

Leishman Eosin Methylene Blue dye solution:

By slowly heating in water bath (40°C), dissolve 0.12 g of Leishman stain powder dye in 100 mL of methanol. Leave it set for 5 days, and then filter.

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.

Diluted Leishman's solution for manual staining

Combine 30mL of Leishman's solution with 150 ml of distilled or demineralized water and with 20 ml of pH 6.8 buffer solution.

Diluted Leishman's solution for automatic staining

Slowly add 30 ml of pH 6.8 buffer solution together with 220 ml of destilled or demineralized water into 50 ml of Leishman's solution. Mix and leave for 10 min.

Blood smear staining procedure using Leishman's solution - manual staining

- Let the smear dry
- Drop the Leishman's solution on the fixed blood smear and let it react for 1 min
- Add 2 mL of buffer solution, gently stir and let it react for 5 min
- Rinse the smear using the pH 6.8 buffer solution
- 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
Thrombocytes - purple
Erythrocytes - reddish

Note

The mentioned formulation is only one of the ways of preparing the dye solution. Alcian Blue 8 GX is most commonly used with Scott and Mowry methods. Depending on personal requests and standard laboratory operating procedures, the dye solution can be prepared according to other protocols.

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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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. Chemicals 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 powder dye in a tightly sealed original packaging at temperature between 15°C and 25°C. Keep in dry places, do not freeze and avoid exposure to direct sunlight. Expiry date is stated 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*; p89, 99-106.
5. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice,* 4th ed., Bloxham, Scion Publishing Ltd.

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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. Medjugorska 59 10040 Zagreb CROATIA 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						