BIOGNOST®

METHYLENE BLUE powder dye, C.I. 52015

IVD *In vitro* diagnostic medical device CE

Basic Blue 9, BSC certified stain

For staining acid-fast bacteria and for staining acc. to Wright

INSTRUCTIONS FOR USE

REF Catalogue number: MB-P-25 (25 g) MB-P-100 (100 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. Methylene Blue powder dye is used in various staining methods in microscopy. It is used for preparation of Romanowsky polychromatic methylene blue and as a part of polychromatic dye for staining bone marrow, animal and herbal material. Methylene Blue is a primary dye for staining acid-fast bacteria acc. to the Ziehl-Neelsen method that uses the Loeffler Methylene Blue reagent.

Product description

- **METHYLENE BLUE** - Biological Stain Commission (BSC) certified powder dye for preparing the solution for staining bacteria acc. to the Breed and Wright methods

Other preparations and reagents used in preparing the dye solution:

- Eosin Y powder dye (product code EOY-P-25, EOY-P-100, EOY-P-500)
- Giemsa powder dye (product code G-P-50)
- 95% methanol (CH_3OH)
- Glycerol (Histanol G, product code HG-1L)
- Acid alcohol (product code KA-OT-1L, KA-OT-2L)
- Absolute ethanol (Histanol 100, product code H100-1L)
- Potassium hydroxide (KOH)
- Buffer solution (pH 6,8 or 7,2)

Preparing the dye for hematology

May-Gruenwald staining solution:

- Mix 0.5 g of Eosin Y powder dye with 0.5 g of Methylene Blue powder dye in 100 mL of distilled/demineralized water.
- Filter. Dry filter. Rinse the residual substance and dry.
- Dilute in 50 mL of methanol.

Giemsa staining solution:

- Dilute 0.76 g of Giemsa powder dye in 50 mL of glycerol.
- Heat for 3 hours in water bath at 60°C.
- Add 50 mL of methanol, let it set for 5 days, then filter it.

Diluted Giemsa solution for staining:

- Dilute 10 mL of Giemsa staining solution with 190 mL of buffer solution.
- Mix well, let it set for 10 minutes, then filter.

Result for hematology

Nucleus - red to purple
Lymphocytes - blue, azure granules (purple to red)
Monocytes - blue
Neutrofil granulocytes - granules of light purple
Eosinophil granulocytes - granules of red to grey-blue
Basophil granulocytes - granules of dark purple
Thrombocytes - purple
Erythrocytes - red
Blood parasites - nuclei (light red)

Preparing the solution for bacteriology staining

1% aqueous potassium hydroxide solution:

- Dissolve 1 g of KOH in 1 liter of distilled/demineralized water by mixing.

Löffler's solution of Methylene Blue powder dye:

- Dissolve 0.3 g of Methylene blue dye with 30 mL of 95% methanol.

Mix with 100 mL of 1% aqueous potassium hidroxyde.

Result for bacteriology

Microbacteria - red
Background - light blue

Note

The mentioned formulation is only one of the ways of preparing the dye solution. Methylene Blue is most commonly used as a part of May-Gruenwald and Giemsa dyes. 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. Both positive and negative controls are recommended before applying.

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 which is available on demand.

Storing, stability and expiry date

Keep Methylene Blue 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. Conn, J. (1977): Biological Stains, 9th ed. Baltimore: Williams and Wilkins Co.
2. Carson, F. L., Hladik, C. (2009): Histotechnology: A Self-Instructional Text, 3rd ed., Chicago: ASCP Press
3. Lillie, R.O. (1969): Biological Stains, 8th ed., Baltimore: Williams & Wilkins Co.
4. Romanowsky, D.L. (1891): St. Petersburg Medizinische Wochenschrift 16: 297-302, 307-315

MB-P-X, V3-EN2, 30 September 2015, VR/IŠP

	Refer to the supplied documentation	°C	Storage temperature range	Σ	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						