BIOGNOST®

METHYL VIOLET 10B powder dye, C.I. 42555

IVD In vitro diagnostic medical device CE

Methyl purple, Gentian violet, Basic violet 3, hexamethyl pararosaniline
For staining bacteria acc. to Gram

INSTRUCTIONS FOR USE

REF Product code: MV10B-P-25 (25 g) MV10B-P-50 (50 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. Methyl Violet 10B powder dye is intended for routine staining according to Gram, for staining bacterial components and for staining amyloids. It is often used for polychromatic section staining in epoxy resins, for viability neuron staining in cell culture, and for meiotic structures analysis.

Product description

- **METHYL VIOLET 10B** - powder dye for preparing solutions for bacterial staining according to Gram.

Example of preparing Methyl Violet 10B dye as Gram staining component

Other preparations and reagents used for perapring dye solutions and with staining process

- Chemicals: ammonium oxalate, iodine, potassium iodide, acetone
- Powder dyes: Safranine O
- 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 or one of more than 30 models of BioGnost's glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm

Preparing dyes solution:

Primary solution

Solution A

- Dissolve 2 g of Methyl Violet 10B powder dye in 20 mL of 95% alcohol solution (Histanol 95).

Solution B

- Dissolve 0.8 g of ammonium oxalate in 80 mL of distilled (demi) water.

Mix solutions A and B, let it set for 24 hours and filter before use.

Gram iodine solution:

- Crush 1 g of iodine and 2 g of potassium iodide in mortar. Slowly add water while continuously stirring until iodine is completely dissolved (add 300 mL of distilled (demi) water in total). Keep in brown glass bottle.

Decolorizing solution:

- Mix 50 mL of acetone and 50 mL of 95% alcohol solution (Histanol 95). Keep in glass bottle.

Counterstain solution

Stock solution

- Dissolve 2.5 g of Safranine O powder dye in 100 mL of 95% alcohol solution (Histanol 95).

Working solution

- Mix 10 mL of stock solution with 90 mL of distilled (demi) water.

Preparing the sample for staining

- Transfer the sample on a clean glass slide using a sterilized smear loop.
  Note: Bodily fluids, discharge, pus, and liquid or solid bacterial culture can be used as samples.
- Spread the sample evenly across the glass slide using 1-2 drops of saline solution.
- Fix the sample using the Bunsen burner after drying by wriggling the glass slide through the cone of flame for 2-3 times.
- Cool the glass slide and begin the process of staining.

NOTE: Apply the reagent so it completely covers the section.

Sample staining procedure

1.	Stain the section using primary solution	**1 min**
2.	Pour excessive dye off the section	
3.	Gently rinse the section under indirect stream of tap water	**2 seconds**
4.	Treat the section using Gram iodine solution	**1 min**
5.	Gently rinse the section under indirect stream of tap water	**2 seconds**
6.	Treat the section with decolorizing solution: end the process when the section turns grey-blue	**10-15 seconds**
	Note: By overly treating with decolorizing solution, the dye will be washed away from Gram-positive bacteria as well	
7.	Stain the section using the counterstain solution	**30 seconds - 1 minute**
8.	Rinse the section carefully with distilled/demineralized water.	**5 seconds**
9.	Dry the section using filter paper or let it dry by air.	
10.	Add a drop of immersion oil on the section (Cedar or Immersion oil).	
11.	Examine the section under immersion lens.	

Result

Gram-positive bacteria - blue-purple
Gram-negative bacteria - red

Note

The mentioned formulation is only one of the ways of preparing the dye solution. Methyl Violet 10B powder dye is most commonly used for staining according to Gram. 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 Methyl Violet 10B 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. 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. Smith, A.C., i Hussey, M.A. (2005): Gram Stain Protocols, American Society for Microbiology Conference for Undergraduate Educators

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Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	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					