BIOGNOST®

METHYLENE BLUE LOEFFLER REAGENT

IVD *In vitro* diagnostic medical device CE

Contrasting blue dye for use in microbiology and histology
Part of TB-Stain Hot kit

INSTRUCTIONS FOR USE

REF Catalogue number: MBL-OT-100 (100 ml) MBL-OT-250 (250 ml) MBL-OT-500 (500 ml) MBL-OT-1L (1000 mL) MBL-OT-2.5L (2500 mL)

Introduction

Methylene Blue Loeffler reagent is used for staining acid fast bacteria according to Ziehl-Neelsen, utilizing the dye's property to stain almost all the bacterial cell's elements in a mildly alkaline medium. This does not apply to acid fast bacteria - they remain stained in their primary color. Methylene Blue Loeffler reagent is suitable for staining and monitoring genera such as Gonococcus, Lactobacillus and for visualizing genus Pasteurella. It is also suitable for staining bacterial nucleus and cytoplasm. Methylene Blue Loeffler reagent is slightly toxic. It can cause cell mutation in very high doses.

Product description

- **METHYLENE BLUE LOEFFLER REAGENT** – Solution for staining in microbiology and histology.

Other slides and reagents that may be used in staining:

- Glass slides used in microbiology, such as VitroGnost ECONOMY GRADE or glass slides used in cytology, such as VitroGnost STANDARD GRADE or high quality glass slides used in histopathology, such as VitroGnost SUPER GRADE or one of more than 30 models of VitroGnost glass slides.
- Primary dye solution for use in staining methods according to Ziehl-Neelsen, such as BioGnost's TB Carbol Fuchsin reagent
- Decolorizer solution for use in staining methods according to Ziehl-Neelsen, such as BioGnost's TB Decolorizer solution
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing the sample for staining

- Transfer the sample on a clean glass slide using a sterilized smear loop.
  Note: Acceptable samples include sputum, lumbar puncture sample, sediment or a histological section.
- 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.
  Note: Samples can be fixated in an oven at temperature 100°C-110°C for 20 min.
- Cool the glass slide and begin the process of staining.

Note: If the sample is a histological section, it should be applied using standard histological techniques.

Sample staining procedure

1.	Cover the samples completely with the TB Carbol Fuchsin reagent. Carefully heat the glass slide containing the sample and dye on the bottom side of the slide using the Bunsen burner until evaporation occurs. Keep the slide hot for 5 min. Do not let the dye boil.	5 min
2.	Rinse with tap water until the dye destains.	
3.	Cover the sample using using TB Decolorizer and let it set for 15-30 seconds (depending on the sample thickness).	15-30 seconds
4.	Rinse with tap water.	
5.	Stain the sample using BioGnost's Methylene Blue Loeffler reagent	30 seconds
6.	Rinse with tap water thoroughly.	
7.	Dry the section	

Results

Acid fast bacteria - red
Background - blue

Note

Microbiology staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out 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 Methylene Blue Loeffler solution in a tightly sealed original packaging at a temperature of +15 to +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. Harvey JW, Keitt AS (May 1983). "Studies of the efficacy and potential hazards of methylene blue therapy in aniline-induced methaemoglobinaemia". Br J Haematol 54 (1): 29-41
2. Madison B (2001). "Application of stains in clinical microbiology". Biotech Histochem 76 (3): 119-25.
3. Margaret A. Bartelt, 2000: Diagnostic Bacteriology: A Study Guide, F.A. Davis Company.

MBL-OT-X, V14-EN5, 30 May 2019, AK/VR

	Refer to the supplied documentation	°C	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						