BIOGNOST®

METHYLENE BLUE LOEFFLER REAGENT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Blue counterstain for use in microbiology and histology

TB-Stain Hot kit component

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC4080299MCKA				
EMDN code	W0104080299				
REF **Catalog number**	**Volume**	**UDI-DI**	REF **Catalog number**	**Volume**	**UDI-DI**
MBL-OT-100	100 mL	03858888820558	MBL-OT-1L	1000 mL	03858890009385
MBL-OT-250	250 mL	03858888820565	MBL-OT-2.5L	2500 mL	03858890009972
MBL-OT-500	500 mL	03858888820572			

Intended use and test principle

Many bacterial cells are easily stained with simple dyes or by the Gram staining method. However, several bacterial genera, such as *Mycobacteria* and *Nocardia*, cannot be stained with simple dyes or, if they are stained, the results vary considerably. The cell walls of the genus *Mycobacteria* contain a waxy substance called mycolic acid. These are β-hydroxy carboxylic acids with chain lengths of up to 90 carbon atoms. The property of acid-fastness is associated with the length of the mycolic acid chain. Staining of such bacterial genera requires either a higher dye concentration or a longer heating period. However, once the bacteria are stained, the dye is even more difficult to remove from the cell. Such bacteria are called acid-fast because they retain the primary stain (Carbol Fuchsin) even after decolorization with acid-alcohol. Early laboratory diagnosis of Tuberculosis is based on the examination of stained smears, and sputum microscopy represents an excellent choice among diagnostic methods. The best-known and still the most widely used method for demonstrating the presence of tuberculosis bacteria is the Ziehl–Neelsen staining method. The Ziehl–Neelsen method uses Carbol Fuchsin as the primary stain, acid-alcohol as the decolorizing agent, and a Methylene Blue solution as the contrast counterstain/background dye. The Methylene Blue Loeffler reagent is a component of BioGnost's TB-Stain HOT kit for Ziehl–Neelsen staining. Its primary role is to stain almost all elements of bacterial cells, other cells, tissues, and the background in a mildly alkaline medium, except for acid-fast bacteria, which remain stained with the primary dye (the TB Carbol Fuchsin reagent).

Product description

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

Example of using Methylene Blue Loeffler reagent as a component of TB-Stain Hot kit

Additional reagents and materials that can be used in the method:

- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Saline solution
- Other components of TB-Stain Hot kit: TB Carbol Fuchsin reagent and TB Decolorizer

Preparation of the sample for staining

- Transfer the sample to a clean glass slide using a sterilized microbiological loop
  Note: The sample can be sputum, punctate sample, or sputum sediment
- Spread the sample evenly on the slide with the help of 1-2 drops of saline solution
- After air drying, fix the sample above the flame of the Bunsen burner by passing the slide briefly through the flame cone 2-3 times
  Note: Samples can also be fixed in an oven at 100 °C to 110 °C for 20 minutes
- Cool the slide and start the staining procedure
  Note: If the sample is a histological preparation, process the sample using standard histological techniques

Sample staining procedure

Note

Apply the reagent so that it completely covers the slide.

1.	Cover the samples completely with the TB Carbol Fuchsin reagent. Carefully heat the slide with the sample and dye from the underside over a Bunsen burner until evaporation, and keep hot for 5 minutes. Do not allow the reagent to boil	**5 min**
2.	Rinse with tap water until the water is discolored	
3.	Immerse the samples in the TB Decolorizer and let it sit for 15-30 seconds (depending on sample thickness).	**15-30 sec**
4.	Rinse with tap water	
5.	Immerse samples in Methylene Blue Loeffler reagent	**30 sec**
6.	Rinse with tap water	
7.	Dry the slide	

It is recommended to use immersion oil during microscopic analysis of stained sample with magnification of 100x.

Result

Acid-fast bacteria - red
Non-fast bacteria, background - blue

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the sample preparation and staining procedure described in this Instruction for use may cause variations 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, sample preparation and staining and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control.
If a serious incident occurs during use 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, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use 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 production date and expiration date are printed on the product 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.

Warnings and precautions regarding the materials contained in the product:	
	H226 Flammable liquid and vapour. 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.

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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		Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
6	Revised acc. to Regulation (EU) 2017/746 - IVDR	08.04.2026