BIOGNOST®

METHYLENE BLUE 10X CONCENTRATE

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

Methylene Blue stock solution for preparing counterstain for acid–fast bacteria

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC4080299MCKA				
EMDN kôd	W0104080299				
REF **Catalog number**	**Volume**	**UDI-DI number**	REF **Catalog number**	**Volume**	**UDI-DI number**
MB10X-OT-100	100 mL	03858890008319	MB10X-OT-500	500 mL	03858890008326
MB10X-OT-250	250 mL	03858892124888	MB10X-OT-1L	1000 mL	03858890001983

Intended use and test principle

Methylene Blue 10X concentrate is used in microbiology, in the Ziehl-Neelsen staining method, where it has a dual function. It serves as a background counterstain for acid-fast bacteria (which are stained red), and also for staining non-acid-fast bacteria, 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 (red). Dilute Methylene Blue 10x concentrate before use. Methylene Blue dye exhibits metachromasia. and it is suitable for staining strains such as *Corynebacterium, Gonococcus, Lactobacillus* and for visualizing *Pasteurella* strain, as well as staining bacterial nuclei and cytoplasm.

Product description

- **METHYLENE BLUE 10X CONCENTRATE** – Stock solution for staining non-acid-fast bacteria acc. to Ziehl-Neelsen

Example of using Methylene Blue 10x concentrate as a component of the TB-Stain HOT kit

Additional reagents and material that can be used in this method

- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade
- The remaining components of TB-Stain HOT kit: TB Carbol Fuchsin reagent, TB Decolorizer

Preparation of Methylene Blue working solution:

- Mix 10 ml of Methylene Blue 10x concentrate solution with 90 ml of distilled/demineralized water. Filter before use.

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 or sputum sediment
- Spread the sample evenly across the glass slide using 1-2 drops of saline solution
- After air-drying, fix the sample by briefly passing the slide through the Bunsen burner 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 glass slide and begin the process of staining.
  Note: If the sample is a histological section, it should be treated and stained 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 **2-10** seconds (depending on the sample thickness).	**2-10 sec**
4.	Rinse in tap water	
5.	Stain using Methylene Blue working solution	**30 sec**
6.	Rinse in tap water thoroughly	
7.	Dry the slide	

It is recommended to use immersion oil for microscopic analysis of the stained sample using magnification of 100x.

Result

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

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the preparation procedure for the working solution and the staining procedure described in these Instructions for Use may cause differences 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. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.

If a serious incident occurs during use of this product 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, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label 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 expiration date is 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 vapor. P210 Keep away from heat, hot surface, sparks, open flames and pther ignition sources. No smoking.. P234 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
4	Revised acc. to Regulation (EU) 2017/746 - IVDR	27.02.2026.