BIOGNOST®

TB MALACHITE GREEN REAGENT

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 - **Class A** product

Green counterstain used when staining acid-fast bacteria

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC4080299MCKA				
EMDN code	W0104080299				
REF **Catalog number**	**Volume**	**UDI-DI**	REF **Catalog number**	**Volume**	**UDI-DI**
TBM-OT-100	100 mL	03858888824327	TBM-OT-1L	1000 mL	03858890009347
TBM-OT-250	250 mL	03858888824334	TBM-OT-2.5L	2500 mL	03858892121795
TBM-OT-500	500 mL	03858888824341			

Intended use and test principle

Many bacterial cells are easily stained with simple dyes or Gram stain. However, several bacterial genera, such as Mycobacteria and Nocardia, cannot be stained with simple dyes or, if stained, the results vary greatly. The cell walls of the genus Mycobacteria contain a waxy substance – mycolic acid. These are β-hydroxy carboxylic acids with chain lengths of up to 90 carbon atoms. The acid-fast property is associated with the length of the mycolic acid chain. Staining of such bacterial genera requires a higher concentration of dye or a longer heating period. However, once the bacteria are stained, the dye is even harder to remove from the cell. Such bacteria are called acid-fast because they retain their primary color (Carbol Fuchsin) even after decolorization with acid alcohol. Early laboratory diagnosis of tuberculosis is based on the examination of stained smears, and an excellent choice among diagnostic methods is sputum microscopy. An alternative to the Ziehl-Neelsen method for detecting the presence of tuberculosis bacteria is the Kinyoun method, which does not require heating of the slide with the sample. The Kinyoun method uses Carbol Fuchsin as the primary stain, acid alcohol as the decolorizing agent, and Malachite Green solution as the counterstain. TB Malachite Green reagent is one of the components of BioGnost's TB-Stain Cold kit, which also contains TB Carbol Fuchsin Reagent and two packages of TB Decolorizer.

Product description

- **TB MALACHITE GREEN REAGENT** – Malachite Green counterstain used when staining acid-fast bacteria

Example of using TB Malachite Green reagent as a component of the TB-Stain Cold 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 the TB-Stain Cold kit: TB Carbol Fuchsin Reagent, 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, a 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 slide, process the sample using standard histological techniques

Sample staining procedure

1.	Immerse the samples in TB Carbol Fuchsin Reagent	**5 min**
2.	Rinse with tap water until the water runs clear	
3.	Immerse the samples in TB Decolorizer and leave for 2-10 seconds (depending on the thickness of the sample)	**15-30 sec**
4.	Rinse with tap water	
5.	Immerse the samples in TB Malachite Green Reagent	**60 sec**
6.	Rinse thoroughly with tap water	
7.	Dry the slide	

Use of immersion oil is recommended during microscopic analysis of stained preparations when using the 100x objective magnification.

Result

Acid-fast bacteria - red
Background - green

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the sample preparation procedure and 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, sample preparation, the staining procedure, 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 or as a result of the use of this product, please report it to the manufacturer and/or authorized representative and the 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 in 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. Madison B (2001). "Application of stains in clinical microbiology". *Biotech Histochem* **76** (3): 119–25.
2. Ryan KJ, Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill.
3. Margaret A. Bartelt, 2000: Diagnostic Bacteriology: A Study Guide, F.A. Davis Company.

Warnings and precautions regarding the materials contained in the product:
Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

TBM-IFU_ENV8, 08.04.2026. IŠP

	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	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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