

TB-STAIN HOT KIT

CE IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Three-reagent kit for staining acid-fast bacteria acc. to Ziehl-Neelsen method

INSTRUCTIONS FOR USE

| | | | | | | | |
|---------------------|-------------------------|---------------|----------------|------------|-----------------------|---------------|----------------|
| BASIC UDI-DI | 385889212HPC4080299MCKA | | | | | | |
| EMDN code | W0104080299 | | | | | | |
| REF | Catalog number | Volume | UDI-DI | REF | Catalog number | Volume | UDI-DI |
| | TBH-100T | for 100 tests | 03858890006186 | | TBH-K-250 | 4x250 mL | 03858888820442 |
| | TBH-K-100 | 4x100 mL | 03858888820435 | | TBH-K-500 | 4x500 mL | 03858888820459 |



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 counterstain. BioGnost's TB-Stain Hot kit consists of the TB Carbol Fuchsin reagent, two packages of TB Decolorizer, and the Methylene Blue Loeffler reagent.

Product description

- **TB-STAIN HOT KIT** - Kit of 3 reagents in 4 packs for staining acid-resistant bacteria according to the Ziehl-Neelsen method

| The kit contains: | for 100 tests (TBH-100T) | 4 x 100 mL (TBH-K-100) | 4 x 250 mL (TBH-K-250) | 4 x 500 mL (TBH-K-500) | Storage temperature |
|---------------------------------|--------------------------|-------------------------|-------------------------|-------------------------|---------------------|
| TB Carbol Fuchsin reagent | 30 mL (TBC-OT-30) | 100 mL (TBC-OT-100) | 250 mL (TBC-OT-250) | 500 mL (TBC-OT-500) | 15-25°C |
| TB Decolorizer | 2 x 30 mL (TBD-OT-30) | 2 x 100 mL (TBD-OT-100) | 2 x 250 mL (TBD-OT-250) | 2 x 500 mL (TBD-OT-500) | 15-25°C |
| Methylene Blue Loeffler reagent | 30 mL (MBL-OT-30) | 100 mL (MBL-OT-100) | 250 mL (MBL-OT-250) | 500 mL (MBL-OT-500) | 15-25°C |

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

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

Note

Apply the reagent so that it completely covers the slide.

Sample staining procedure

a) using kit for 100 tests (TBH-100T)

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| 1. | Apply TB Carbol Fuchsin reagent (≥ 5 drops). 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. | Apply TB Decolorizer and leave it for 15-30 seconds (depending on sample thickness) (≥ 5 drops) | 15-30 sec |
| 4. | Rinse with tap water | |
| 5. | Apply Methylene Blue Loeffler reagent (≥ 5 drops) | 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

b) using three-reagent kit of 100, 250 and 500 mL (TBH-K-100, TBH-K-250, TBH-K-500)

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|----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1. | Cover the slides 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
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 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, staining procedure and diagnosis can only be carried out by authorized and professionally qualified persons. 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 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

- Ziehl, F. (1882): Zur Färbung des Tuberkelbacillum. Deutsche Medizinische Wochenschrift, V8, p. 451.
- Neelsen, P. (1883): Zentralblatt für die Medizinischen Wissenschaften, V21, p. 497
- Madison, B. (2001): Application of stains in clinical microbiology. Biotech Histochem 76 (3): 119–25.
- Ryan, K.J., Ray, C.G. (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill.

| Warnings and precautions regarding the materials contained in the product: | |
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|  | <p>H225 Highly flammable liquid and vapour. H226 Flammable liquid and vapour. H314 Causes severe skin burns and eye damage. H301 Toxic if swallowed. H311 Toxic in contact with skin. H331 Toxic if inhaled. H341 Suspected of causing genetic damage. H373 May cause damage to organs through prolonged or repeated exposure.</p> |
| | <p>P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources . No smoking.</p> |
| | <p>P233 Keep container tightly closed.</p> |
| | <p>P280 Wear protective gloves/protective clothing/eye protection/face protection.</p> |
| | <p>P302+P352 IF ON SKIN: wash with plenty of water.</p> |
| | <p>P308+P311 IF exposed or concerned: Call a POISON CENTER or doctor/physician.</p> |
| | <p>P305+P351+P338 IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.</p> |
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TBH-IFU_ENV13, 08.04.2026, IŠP

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|  Manufacturer |  Batch code |  Consult Instructions for use |  Contains sufficient for <n> tests |  Contains hazardous substances |
|  Date of manufacture |  Catalogue number |  Caution |  European conformity |  Fragile, handle with care |
|  Use-by date |  Temperature limit |  <i>In vitro</i> diagnostic medical device |  Unique device identifier | |

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| Version | Description / reason for change | Date |
|---------|-------------------------------------------------|------------|
| 13 | Revised acc. to Regulation (EU) 2017/746 - IVDR | 08.04.2026 |