

TB-STAIN HISTO KIT

IVD *In vitro* diagnostic medical device



Three-reagent kit for staining acid-fast bacteria in histology sections according to Ziehl-Neelsen

INSTRUCTIONS FOR USE

REF Catalogue number: TBHK-100T (for 100 tests)

Introduction

Many bacterial cells are easily stained by using simple dyes or Gram stain. However, a few bacterial strains, such as *Mycobacteria* and *Nocardia* cannot be stained using simple dyes (or, if successfully stained, the results may vary significantly). Cellular wall of the *Mycobacteria* strain contains waxy substance - mycolic acid. Those are beta-hydroxy carboxylic acids with chains containing up to 90 carbon atoms. Its resistance to acidity is associated with mycolic acid chain length. In order to stain such strains, a higher concentration of dye or a longer period of heating is required. However, once stained, the dye is even more difficult to remove from the cells. Those bacteria are called acid resistant because they maintain their primary color even after decolorization using acid alcohol (Carbol Fuchsin). Early laboratory diagnosis of tuberculosis is based on the interpretation of stained smears, and one of the best diagnostic methods is analyzing sputum sample under microscope. The most common and renowned method used for detecting the tuberculosis bacteria is staining according to Ziehl-Neelsen. This method uses Carbol Fuchsin as the main dye, acid alcohol as decolorization medium and Methylene Blue solution as contrasting dye. BioGnost's TB-Stain Hot kit contains TB Carbol Fuchsin reagent, two packages of TB Decolorizer and Methylene Blue Loeffler reagent.

Product description

- **TB-STAIN HISTO KIT** - Three-reagent kit in 4 packages. For staining acid-fast bacteria in histology sections according to Ziehl-Neelsen.

The kit contains:	for 100 tests (TBHK-100T)
TB Carbol Fuchsin reagent	30 mL (TBC-OT-30)
TB Decolorizer	2 x 30 mL (TBD-OT-30)
Methylene Blue Loeffler reagent	30 mL (MBL-OT-30)

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 52/54, BioWax 56/68, BioWax Blue, BioWax Micro.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μ m slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Add TB Carbol Fuchsin reagent (≥ 5 drops)	30 min
	Note: place the section in an incubation jar in order to avoid drying of the section	
6.	Rinse in distilled (demi) water and dry using filter paper	
7.	Add TB Decolorizer (≥ 5 drops)	5-15 seconds
8.	Rinse under tap water	3 min
9.	Add Methylene Blue Loeffler reagent (≥ 5 drops)	30 seconds
10.	Rinse in distilled (demi) water	3 exchanges, 10 seconds each
11.	Rinse in tap water	2 min
	Note: in order to make the stain intensity greater, the section can immediately be covered with BioMount Aqua reagent and VitroGnost cover glass	
12.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
13.	Dehydrate using 100% alcohol (Histanol 100)	2 min
14.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Washout of dye during dehydration process is avoided by using BioMount Aqua; this way more consistent results can be achieved. In case of dehydration and clearing, apply an appropriate BioMount medium for mounting the cover glass. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

Results

Acid fast bacteria - red
 Nuclei and 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.

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 the TB-Stain Histo kit in a tightly sealed original packaging at temperature of 15°C 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. Ziehl, F. (1882): Zur Färbung des Tuberkelbacillum. Deutsche Medizinische Wochenschrift, V8, p 451.
2. Neelsen, P. (1883): Zentralblatt für die Medizinischen Wissenschaften, V21, p 497
3. Madison, B. (2001): Application of stains in clinical microbiology. Biotech Histochem 76 (3): 119-25.
4. Ryan, K.J., Ray, C.G. (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill.

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				



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