BIOGNOST®

TB-STAIN ECO KIT

IVD In vitro diagnostic medical device CE

Three reagent kit for staining acid fast bacteria according to modified Ziehl-Neelsen method, phenol-free

INSTRUCTIONS FOR USE

REF Product code: TBE-K-100 (4x100 mL) TBE-K-250 (4x250 mL) TBE-K-500 (4x500 mL)

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 fast because they maintain their primary color even after decolorization using acid alcohol (TB Fuchsin reagent, phenol-free). 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 kit uses modified Ziehl-Neelsen method that contains TB Fuchsin reagent, phenol-free, acid alcohol as decolorizing agent (two packages of TB Decolorizer) and Methylene Blue solution as counterstain (Methylene Blue Loeffler reagent).

Product description

- **TB-STAIN ECO KIT** - Three-reagent kit in 4 packages. For staining acid fast bacteria, phenol-free.

The kit contains:	4x100 mL (TBE-K-100)	4x250 mL (TBE-K-250)	4x500 mL (TBE-K-500)
TB Fuchsin reagent	100 mL (TBFR-OT-100)	250 mL (TBFR-OT-100)	500 mL (TBFR-OT-500)
TB Decolorizer	2 x 100 mL (TBD-OT-100)	2 x 250 mL (TBD-OT-100)	2 x 500 mL (TBD-OT-500)
Methylene Blue Loeffler reagent	100 ml (MBL-OT-100)	250 mL (MBL-OT-100)	500 ml (MBL-OT-500)

Other sections and reagents that may be used in staining:

- 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 microbiology sections for staining

Other preparations and reagents that may be used:

- Glass slides used in microbiology, such as VitroGnost ECONOMY GRADE or glass slides used in cytology, such as VitroGnost STANDARD GRADE or high quality glass slides used in histopathology, such as VitroGnost SUPER GRADE or one of more than 30 models of VitroGnost glass slides.
- BioGnost's immersion oils, such as Immersion oil and Immersion oils types 37, A, or FF

Preparing the sample for staining

- Transfer the sample on a clean glass slide using a sterilized smear loop.
  Note: Bodily fluids, discharge, pus, and liquid or solid bacterial culture can be used as samples.
- Spread the sample evenly across the glass slide using 1-2 drops of saline solution.
- After drying on air, fix the sample using the Bunsen burner by wriggling the glass slide through the cone of flame for 2-3 times. Cool the glass slide and begin the process of staining.
- The sample may be fixed by adding a few drops of methanol to the section. Let it act for 1-2 minutes and continue with staining.

NOTE

Apply the reagent so it completely covers the section.
Bring TB Fuchsin reagent to a temperature of +80°C to +90°C.

Sample staining procedure

1.	Immerse the samples completely in preheated TB Fuchsin reagent.	30 min
	Note: place the section in an incubation jar in order to avoid drying of the section	
2.	RInse in distilled (demi) water and dry using filter paper	
3.	Cover the sample using using TB Decolorizer and let it set for 15-30 seconds (depending on the sample thickness).	5-15 seconds
4.	Rinse under tap water	3 min
5.	Stain the sample using BioGnost's Methylene Blue Loeffler reagent	30 seconds
6.	Rinse in distilled (demi) water	3 exchanges, 10 seconds each
7.	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	

Washout of dye during dehydration process is avoided by using BioMount Aqua; this way more consistent results can be achieved. Cover the section with VitroGnost cover glass.

Result

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 TB-Stain Eco 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 Farbung des Tuberkelbacillum. Deutsche Medizinische Wochenschrift, V8, p 451.
2. Neelsen, P. (1883): Zentralblatt fur de Medizinischen Wissenschafen, 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.

TBE-X, V2-EN2, 15 November 2019, MAS/IŠP

Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
Refer to supplied instructions	Keep away from heat and sunlight	Valid until	LOT	Lot number	Manufacturer		
IVD For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile					