

TB FUCHSIN REAGENT

IVD *In vitro* diagnostic medical device



For use in TB-STAIN ECO kit

INSTRUCTIONS FOR USE

REF Product code: TBFR-OT-100 (100 mL) TBFR-OT-250 (250 mL) TBFR-OT-500 (500 mL) TBFR-OT-1L (1000 mL)

Introduction

TB Fuchsin reagent is a component of TB-Stain ECO kit in which it dyes acid-fast bacteria. 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. TB-Stain ECO kit uses modified Ziehl-Neelsen method that contains TB Fuchsin reagent, phenol-free, acid alcohol as decolorizing agent and Methylene Blue solution as counterstain. BioGnost's TB-Stain Eco kit contains TB Carbol Fuchsin, phenol-free reagent, two packages of TB Decolorizer and Methylene Blue Loeffler reagent.

Product description

- **TB FUCHSIN REAGENT** – Basic Fuchsin dye solution, phenol-free

Example of use of TB Fuchsin reagent as a component of TB-Stain ECO kit

Other sections and reagents that may be used in staining:

- 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
- BioGnost's reagents: TB Decolorizer (TBD-OT-100, TBD-OT-250, TBD-OT-500), Methylene Blue Loeffler reagent (MBL-OT-100, MBL-OT-250, MBL-OT-500)

Preparing microbiology sections 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.

Sample staining procedure

1.	Heat the samples briefly at 80-90°C in thermostat.	
2.	Apply TB Fuchsin reagent and incubate in thermostat at 80-90°C. Note: If the section gets dried, during incubation add additional amount of TB Fuchsin reagent	3-4 minutes
3.	Rinse the sections using a dropper with 5 ml of distilled (demi) water	10 seconds
4.	Cover the samples with TB Decolorizer.	15 seconds
5.	Rinse the sections using a dropper with 5 ml of distilled/demi water.	10 seconds
5.	Stain with Methylene Blue Loeffler reagent	2-3 minutes
6.	Rinse the section with a dropper with 3-5 ml of distilled (demi) water	
7.	Let the sections set on air	

Add a drop of immersion oil to the dried section and observe under immersion lens.

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 Fuchsin reagent 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, p451.
2. Neelsen, P. (1883): Zentralblatt für die Medizinischen Wissenschaften, V21, p497
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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