TB-STAIN HOT KIT

IVD *In vitro* diagnostic medical device CE

Three reagent kit for staining acid fast bacteria according to Ziehl-Neelsen
INSTRUCTIONS FOR USE

REF Catalogue number: TBH-K-100 (4x100 ml) TBH-K-250 (4x250 ml) TBH-K-500 (4x500 ml)

Introduction

Many bacterial cells are easily stained by using simple dyes or Gram stain. However, a few strains of bacteria, such as *Mycobacteria* and *Nocardia* cannot be stained using simple dyes (the results may vary significantly if successfully stained). 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 (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 HOT** - Three-reagent kit in 4 packages. For staining acid fast bacteria according to Ziehl-Neelsen.

The kit contains:	4 x 100 mL (TBH-K-100)	4 x 250 mL (TBH-K-250)	4 x 500 mL (TBH-K-500)
TB Carbol Fuchsin reagent	100 ml (TBC-OT-100)	250 ml (TBC-OT-250)	500 ml (TBC-OT-500)
TB Decolorizer	2 x 100 mL (TBD-OT-100)	2 x 250 mL (TBD-OT-250)	2 x 500 mL (TBD-OT-500)
Methylene Blue Loeffler reagent	100 ml (MBL-OT-100)	250 ml (MBL-OT-250)	500 ml (MBL-OT-500)

Other slides and reagents that may be used in staining:

- 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 media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing the sample for staining

- Transfer the sample on a clean glass slide using a sterilized smear loop.
  Note: Acceptable samples include sputum, lumbar puncture sample or a sputum sediment.
- Spread the sample evenly across the glass slide using 1-2 drops of saline solution.
- Fix the sample using the Bunsen burner after drying by wriggling the glass slide through the cone of flame for 2-3 times.
  Note: Samples can be fixated in an oven at temperature 100°C-110°C for 20 min.
- Cool the glass slide and begin the process of staining.
  Note: If the sample is a histological section, it should be processed using standard histological methods.

Sample staining procedure

1.	Cover the samples completely with the TB Carbol Fuchsin reagent. Carefully heat the glass slide containing the sample and dye on the bottom side of the slide using the Bunsen burner until evaporation occurs. Keep the slide hot for 5 min. Do not let the dye boil.	5 min
2.	Rinse with tap water until the dye destains.	
3.	Cover the sample using using TB Decolorizer and let it set for 15-30 seconds (depending on the sample thickness).	15-30 seconds
4.	Rinse with tap water.	
5.	Stain the sample using BioGnost's Methylene Blue Loeffler reagent	30 seconds
6.	Rinse with tap water thoroughly.	
7.	Dry the section	

Results

Acid fast bacteria - red
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 Hot 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.

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Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	CE	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						