

TB PERMANGANATE REAGENT

IVD In vitro diagnostic medical device

 ϵ

For use in TB-Stain Fluorescent kit **INSTRUCTIONS FOR USE**

REF Catalogue number: TBP-0T-100 (100 ml) TBP-0T-250 (250 ml)

TBP-0T-500 (500 ml)

TBP-0T-1L (1000 mL)

TBP-OT-2.5L (2000 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). Cell walls of the Mycobacteria strain contain fatty acids which make them hard to stain. In order to stain the walls, a higher concentration of dye or a longer period of heating is required. However, once stained, the dye is ever more difficult to remove from the cells. Those bacteria are called acid fast because they remain their primary color even after being treated with acid alcohol (3% HCl alcohol solution). Fluorescence has been used to detect acid fast bacteria for many years. This method is more sensitive than the Kinyoun method. It takes less time to interpret the results. Auramine-Rhodamine, acid alcohol (0.75% HCl alcohol solution) as a differentiation medium and potassium permanganate as a contrasting dye are used in this method.

Product description

TB PERMANGANATE REAGENT - Contrasting dye for detection of acid fast bacteria using the fluorescence method.

Other slides and reagents that may be used in staining:

- 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
- 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.
- 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
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- TB Decolorizer Fluorescence for use with the TB-Stain Fluorescence kit, product code TBF-OT-100; TBF-OT-110, TBF-OT-250; TBF-OT-500
- Primary dye, such as TB Auramine-Rhodamine reagent for use with the TB-Stain Fluorescent kit. -

Preparing the section for staining

SPUTUM

- The sputum sample must be treated with a preparation containing hypochlorite in order to isolate the mycobacteria from the surrounding mucus. LUMBAR PUNCTION SAMPLES, SEDIMENTS
- After finishing the process of enrichment, smear the sample on the glass slide and let it dry.

HISTOLOGICAL SECTIONS

- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioNene, BioClear New).
- Rehydrate the section through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80, and Histanol 70). Note: preparation of the section depends on the type of the section. Preparation is not necessary for all types of samples. The samples must be fixated using heat using Bunsen burner or oven.

Sample staining procedure

| Solution | Preparation time (min) |
|------------------------------|------------------------|
| Auramine-Rhodamine | 15 |
| Rinse with tap water | 10 |
| TB Decolorizer fluorescent | 1 |
| Rinse with tap water | 5 |
| Potassium permanganate | 5 |
| Rinse with tap water and dry | 5 |

Acid fast bacteria - red-orange or yellow-green (depends on filters used on microscope) Background - black

Time periods of staining procedures are not standardized. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 Permanganate reagent in a tightly closed original package at temperature of $+15^{\circ}$ C to $+25^{\circ}$ 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. Madison B (2001). "Application of stains in clinical microbiology". Biotech Histochem 76 (3): 119-25.
- 2. Ryan KJ, Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill.
- 3. Margaret A. Bartelt, 2000: Diagnostic Bacteriology: A Study Guide, F.A. Davis Company.

TBP-OT-X, V5-EN3, 28 May 2019, IŠP/VR

| <u> </u> | Refer to the supplied documentation | °C-¶-°C | Storage temperature range | \sum | Number of tests in package | REF | Product code | (| ∶€ | European Conformity | *** | BIOGNOST Ltd. Medjugorska 59 10040 Zagreb | (| ϵ |
|----------|-------------------------------------|----------|-------------------------------------|--------|----------------------------------|-----|---------------|---|----|------------------------|-----|---|---|------------|
| []i | Refer to supplied instructions | 类 | Keep away from heat and sunlight | \Box | Valid until | LOT | Lot number | 1 | 5 | Manufacturer | | CROATIA www.biognost.com | | |
| IVD | For in vitro diagnostic use only | * | Keep in dry place | 4 | Caution - fragile | | | | | | | | | |