GABBETT REAGENT

IVD *In vitro* diagnostic medical device CE

Blue counterstain for staining acid-fast bacteria according to Kinyoun
INSTRUCTIONS FOR USE

REF Catalogue number: GAB-OT-100 (100 mL) GAB-OT-250 (250 mL) GAB-OT-500 (500 mL) GAB-OT-1L (1000 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 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. Method according to Kinyoun is an alternative to the Ziehl-Neelson method of detecting tuberculosis bacteria. The Kinyoun method does not require heating the glass slide containing the sample. Kinyoun method uses Carbol Fuchsin as the main dye, acid alcohol as decolorization medium and TB Malachite Green solution as counterstain. Instead of TB Malachite Green reagent, Gabbett reagent may also be used, as it contains Methylene blue dye.

Product description

- **GABBETT REAGENT** – Blue counterstain for staining acid-fast bacteria.

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.
- Primary dye solution for use in staining methods according to Ziehl-Neelsen or Kinyoun, such as BioGnost's TB Carbol Fuchsin reagent or TB Carbol Fuchsin Kinyoun reagent.
- Decolorizer solution for use in staining methods according to Ziehl-Neelsen or Kinyoun, such as BioGnost's TB Decolorizer solution
- Counterstain solution for use in staining methods according to Ziehl-Neelsen, such as BioGnost's Methylene Blue Loeffler reagent.
- Immersion oils, such as BioGnost's Immersion oil, Cedarwood oil, Immersion oils types A, B, NVH, FF and 37

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, sediment or a histological section.
- 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 applied using standard histological techniques.

Sample staining procedure according to Kinyoun

1.	Cover the samples completely with the TB Carbol Fuchsin Kinyoun reagent.	20 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.	Stain with Gabbett reagent	5 min
5.	Rinse with tap water thoroughly.	
6.	Dry the section.	10-15 seconds

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. 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 Gabbett reagent in a tightly sealed original packaging at temperature between 15 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. Madan, M., Ranjitham, M., Laksham, C. (1999): Cold Staining Method for Acid Fast Bacilli, *Ind. J. Pathol. Microbiol.* 42(4): 505-507
2. Tan Thiam Hok (1962): Simple and rapid Cold Satining Method for Acid Fast Bacteria, *Am. Rev. Resp. Disc.;* 85,753
3. Vasantha K.R., Jagannath,K., Rajasekaran, S. (1986): A Cold Staining Method For Acid Fast Bacilli, *Bull.* W.H.O., 64 (5), 741.

GAB-OT-X, V2-EN2, 21 February 2017, AK/VR

	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity
	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				

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