todylaboratories®

**Microbiology Stains**

CE IVD REF C15MS1087

# Methylene Blue 10X Concentrate Instructions For Use

Methylene Blue stock solution for use in microbiology
*In vitro* diagnostic medical device

## INTRODUCTION

Methylene Blue 1ox concentrate is used for staining acidoresistant bacteria according to Ziehl-Neelsen, utilizing the dye's property to stain almost all the bacterial cell's elements in a mildly alkaline medium. This does not apply to acid-fast bacteria - they remain stained in their primary color. Dilute Methylene Blue 10x concentrate before use. Methylene Blue dye indicates metachromasia and it is suitable for staining strains such as *Corynebacterium*, *Gonococcus*, *Lactobacillus* and for visualizing *Pasteurella* strain, as well as staining bacterial nuclei and cytoplasm.

## PRODUCT DESCRIPTION

METHYLENE BLUE 10X CONCENTRATE – Stock solution for staining in microbiology

## 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, such as TB Carbol Fuchsin reagent
- Decolorizer solution for use in staining methods according to Ziehl-Neelsen, such as TB Decolorizer solution
- Immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

## PREPARATION OF SOLUTIONS

**Working Methylene Blue (Loeffler) solution**

- Mix 10 ml of Methylene Blue 10x concentrate solution with 90 ml of distilled (demineralized) water.

## 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.

- Spreadthesampleevenlyacrosstheglassslideusing1-2dropsofsalinesolution.
- 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

| | | |
|---|---|---|
| 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 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. 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 the material safety data sheet.

## STORING, STABILITY AND EXPIRY DATE

Keep Methylene Blue 10X concentrate in a tightly sealed original packaging at temperature between 15°C and 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. Harvey JW, Keitt AS (May 1983). "Studies of the efficacy and potential hazards of methylene blue therapy in aniline-induced methaemoglobinaemia". Br J Haematol 54 (1): 29-41
2. Madison B (2001). "Application of stains in clinical microbiology". Biotech Histochem 76 (3): 119-25.
3. Margaret A. Bartelt, 2000: Diagnostic Bacteriology: A Study Guide, F.A. Davis Company.


## SYMBOL INDEX

| | |
|---|---|
| | Manufacturer |
| IVD | For in vitro diagnostic use only |
| LOT | Lot number |
| | Valid until |
| | Storage temperature range |
| | Keep away from heat and sunlight |
| CE | European Conformity |
| REF | Product code |
| | Refer to supplied instructions |
| Σ | Number of tests in package |
| | Refer to the supplied documentation |
| | Caution - fragile |
| | Keep in dry place |

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