Methylene Blue

Cat. 4603

For staining microorganisms by Ziehl-Neelsen acid-fast procedure.For “in vitro” diagnostic.

Practical information

Aplications	Categories
Stain reagents	Acid-fast organisms (AFB)

Industry: Dyes and stains

Principles and uses

The acid-fast stain is a differential stain.

Bacteria are classified as acid-fast if they retain the primary stain (carbol fuchsin) after washing with strong acid and appear red, or as non-acid-fast if they lose their colour on washing with acid and counter stained by the methylene blue. Acid-fast property is due to the presence of high contents of a lipid called mycolic acid in the cell wall, that makes penetration by stains extremely difficult. Once the stain has penetrated it cannot be readily removed.

Formula in g/L

Ethanol	300	Methylene blue	5
Phenol	10	Water	685

Instructions for use

1. Place slides on a staining rack and place a piece of filter paper, larger than the size of the smear, on each slide.
2. Flood with carbol fuchsin. Heat gently to steaming and allow to steam for 5 min. Do not overheat.
3. Remove the filter paper.
4. Wash gently in running water.
5. Decolorize with a decolorizer with two changes of reagent for 1-2 min until no more red color appears in washing.
6. Wash slides gently in running water.
7. Counterstain with methylene blue for 30 s.
8. Wash gently in running water.
9. Dry over gentle heat.
10. Examine under a microscope.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Liquid	N/A	N/A	N/A

Microbiological test

Note: Any interference is not known. Acid, basic or high levels of Chloride or salts in wash water could alter the results.

Microrganisms	Characteristic reaction
Acid-fast organisms (AFB)	Orange-red colonies
Non-acid-fast organisms	Blue colonies

Storage

Temp. Min.:15 ºC
Temp. Max.:30 ºC

Bibliography

Truant, Brett, Thomas, fluorescent microscopy acid-fast procedure 1962, 382-383 in Clarck, G., Staining procedures (1981), 4th ed. W&W.
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Swamy, P. (2009). Laboratory Manual on Biotechnology. New Delhi: Rastogi Publications.