



Malachite Green 1% (SI044)

Use

Malachite Green 1% is used as staining solution in spore staining and simple staining.

Principle

Malachite Green is used for bacterial spore staining by Schaeffer and Fulton's method. Vegetative cells are then counterstained with Safranin. Spores may be located in the middle of the cell, at the end of the cell or between the end and middle of the cell. Spores may be spherical or elliptical.

A **differential staining** technique (the Schaeffer-Fulton method) is used to distinguish between the vegetative cells and the endospores. A **primary stain (malachite green)** is used to stain the endospores. Because endospores resist staining, the malachite green will be **forced** into (*i.e. malachite green permeate the spore wall*) the endospores by **heating**. In this technique heating acts as a **mordant**.

There is no need of using any decolorizer in this spore staining as the primary dye malachite green binds relatively weakly to the cell wall and spore wall. In fact, if washed well with water the dye comes right out of the cell wall, however not from the spore wall once the dye is locked in. **Water is used to decolorize** the vegetative cells.

As the endospores are resistant to staining, the endospores are equally resistant to de-staining and will retain the primary dye while the vegetative cells will lose the stain. The addition of a counterstain or **secondary stain (safranin)** is used to stain the decolorized vegetative cells.

When visualized under microscopy the cells should have three characteristics:

1. The vegetative cells should appear pink/red (*i.e.* color of counter stain),
2. The vegetative cells that contain endospores should stain pink while the **spores should be seen as green** ellipses within the cells.
3. Mature, free endospores should not be associated with the vegetative bacteria and should be seen as green ellipses.

Formula

Ingredients	Formula / Litre
Malachite green	1.000 gm
Distilled water	100.000 ml

Storage And Stability

1. Store the bottle in dry, cool and dark place.
2. The shelf life of reagents is as per the expiry date mentioned on the reagent bottle labels.

Procedure

- 1) Prepare a smear on a clear, dry glass slide.
- 2) Allow it to air dry and fix it with gentle heat.
- 3) Flood the slide with 1% Malachite Green (SI044).
- 4) Allow the stain to be in contact with the smear for 2-3 minutes and heat the preparation for 3-6 minutes and then allow to cool.
- 5) Wash in slow-running tap water.
- 6) Counterstain with 0.5% aqueous safranin (SI028) for 30 seconds.
- 7) Wash with water, blot dry and examine under oil immersion objective.

PRODUCT SPECIFICATION SHEET



Result

Microscopic examination:

Metachromatic staining was carried out by Schaeffer and Fulton's method and staining characteristics of the organism is observed under microscope by using oil immersion lens.

The vegetative cells will appear pink/red and the spores will appear green.

Packaging

Product Name : **Malachite Green 1%**

Product Code : **SI044**

Available Pack sizes : **100ml**

Further Information

For further information please contact your local MICROMASTER Representative.



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