



Nigrosin Stain,10% w/v

S025

Intended use

Nigrosin stain, 10% w/v is used as staining solution for negative staining.

Composition**

Ingredients

10.0 gm
0.5 gm
100.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. To a loopful of cerebrospinal fluid, or to a light aqueous or saline suspension of growth from an agar culture, add a loopful of Nigrosin (S025).

2. Mix well and cover with a thin cover glass. If only a few organisms are present, centrifugation of the cerebrospinal fluid may be necessary.

3. Examine promptly with a high power lens. Light may have to be reduced by lowering the condenser. Oil immersion may be used, if higher magnification is required.

Principle And Interpretation

Negative staining is one of the many staining techniques that can be employed for viewing of bacterial cell morphology and size. The advantages of the negative stain include the use of only one stain and the absence of heat fixation of the sample. Negative staining employs the use of an acidic stain and, due to repulsion between the negative charges of the stain and the bacterial surface, the dye will not penetrate the cell. In negative staining, the results yield a clear cell with a dark background. Negative staining method also permits visualization of the usually transparent and unstainable capsule of many organisms, most importantly *Cryptococcus neoformans*. Nigrosin is used for negative Staining of bacteria, as well as the capsule-containing fungus, *Cryptococcus neoformans*. Nigrosin consists of a suspension of fine particles of carbon. These form a dark background, against which capsules are clearly seen as a result of displacement of the carbon particles. The shapes and sizes of the organisms are seen as color-free outlines against the dark background. An advantage of using this method, rather than regular positive stains like methylene blue or carbol fuchsin, is that prior fixation by heat or alcohol is not needed, so the organisms are seen in more lifelike shapes. Furthermore, negative staining with nigrosin can reveal some microorganisms that cannot be stained by regular methods.

Type of specimen

Clinical samples; food & dairy samples; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines . For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye

Limitations

- 1. Negative staining does not differentiate bacteria, one can only determine morphology.
- 2. Certain areas might acquire more stain and therefore appear with higher contrast than would be normal.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- → **Appearance :** Blackish violet coloured solution.
- \rightarrow Clarity : Clear without any particles.
- → Microscopic Examination : Negative staining is carried out. Staining characteristics of organism i s observed under microscope by using oil immersion lens
- → **Results :** Clear halos surrounding the bacterial cells

Storage and Shelf Life

Store between 10- 30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.

10° <u>C</u>	Storage temperature	8	Do not use if package is damaged
IVD	In vitro diagnostic medical device	CE	CE Marking
	HiMedia Laboratories Pvt. Limited, C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W) - 400604, MS, India	ECREP	CEpartner4U,ESDOORNLAAN 13,3951 DB MAARN,The Netherlands, www.cepartner4u.eu

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HiMedia Laboratories Pvt. Ltd. Reg.Office : Plot No:C-40, Road No: 21Y, MIDC, Wagle IndustrialPaArea, Thane(West)-400604, Maharashtra, INDIA.Tel:00-91-22-61471919/61169797/69034800, Fax:00-91-22-61471920.Email : techhelp@himedialabs.com