



PRODUCT SPECIFICATION SHEET

NITRATE REAGENT-I (SULPHANILIC ACID-0.8%) NITRATE REAGENT-II (NAPHTHYLAMINE SOLUTION)

Use

Nitrate Reagents A & B are intended for use in the detection of nitrate reduction by aerobic and anaerobic bacteria.

Principle

Many aerobic and anaerobic bacteria use nitrate as a source of nitrogen, but before it can be used, it must be reduced. Organisms that have the enzyme nitrate reductase are capable of reducing nitrate to nitrite. Other organisms are capable of reducing nitrite to nitrogen gas.

For the detection of nitrate reduction, the organism grown in a Nitrate broth(DM179) containing 0.2% Potassium nitrate. If the organism produces nitrate reductase, nitrate will be broken down into a colorless product. Sulphanilic Acid(Reagent A) is added to create an acid solution. Then add Alpha-naphthylamine(Reagent B).If nitrites are produced, the reagent will react with the nitrites to form a diazonium compound, resulting red in color. If no color change occurs, either the organism does not produce the enzyme, or the nitrates have been reduced to nitrogen. If nitrogen is produced, no color change will occur with the addition of zinc dust. If nitrate is still present in the medium, indicating the absence of the enzyme, zinc dust react with the nitrate to produce a red color.

Formula

Ingredients	Formula / Litre
Nitrate Reagent A	
Sulphanilic Acid	8.0 gm
Acetic acid 5N	1000 ml
Nitrate Reagent B	
N,N-Dimethyl-1-Naphthylamine	6.0ml
Acetic acid 5N	1000ml

Reagent Storage And Stability

- 1.Storage: Upon receipt, store at room temperature (10°C - 30°C) in original packaging until use. Do not use the product if there are signs of deterioration or contamination. The expiration date applies to the product in its original packaging and stored as directed.
2. The shelf life of reagents is as per the expiry date mentioned on the reagent bottle labels.

Precautions

1. For Invitro Diagnostic use only.
2. Observe all standard safety precautions consistent with hazard(s) stated.
3. Approved biohazard precautions and aseptic techniques should be observed when using this product. This product is for use only by properly-trained and qualified personnel. Sterilize all biohazard waste prior to disposal.

Procedure

1. Specimen Collection: Not applicable since these reagents are used in media which are not for primary isolation. So direct inoculation of characterizing cultures of isolated organisms into the media to which these reagents are added will produce erroneous results
2. Inoculate appropriate media containing nitrate(DM179Nitrate Broth or DM121 Indole- Nitrate Medium)
3. Incubate aerobically at 30-35°C for 18-24hrs.Examine the gas buubles.
4. After incubation, add equal parts of regent A and B.
5. Production of a pink color indicates the production of nitrites. If no color change occurs, the tube may be re-incubated and retested.



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6. If gas has been produced, add the reagents. If no color change occurs, add the reagents as described above, If no color change results, add zinc dust. If the solution remains colorless, the nitrate has been reduced to nitrogen gas and ammonia and considered a positive test.

Limitations

Nitrate reduction reagents will not provide complete information for the identification of bacterial isolates. Additional test procedures and media are required for complete identification. Consult reference materials for additional information.

Interpretation

Positive: A positive reaction is indicated by the production of a red color after addition of Nitrate reagent A and B and after the addition of zinc dust (Nitrates reduced to nitrogen and ammonia).

Negative: Negative reaction indicated by no color change after the addition of Nitrate Reagents A and B and red color after the addition of zinc dust.

User Quality Control

The following test strains are recommended:

Organism	Expected Results	
<i>Pseudomonas aeruginosa</i> ATCC 27853	Positive	Red color
<i>Proteus mirabilis</i> ATCC12453	Positive	Red color
<i>Escherichia coli</i> 25922	Positive	Red color
<i>Acinetobacter baumannii</i> ATCC19606	Negative	No red color

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If sterility testing is to be performed on this product, the appropriate percentage of the original shipment amount should be incubated anaerobically and aerobically for 48 - 96 hours.

If the reactivity capacity of this reagent is to be tested for performance, it is recommended that the following ATCC organisms be evaluated, using Anaerobic Brucella Blood Agar plates, for reactivity.

Organism Expected

Physical Appearance

Nitrate A Reagent should appear as a clear yellowish liquid within the bottle. The Nitrate B Reagent should appear as a clear, pinkish liquid within the bottle.

Packaging

Product Name : Nitrate Reagent

Product Code : IR004

Available Pack sizes : 100ml

Storage & Stability

Storage: Upon receipt, store at room temperature (13°C - 27°C) in original packaging until use. Do not use the product if there are signs of deterioration or contamination. The expiration date applies to the product in its original packaging and stored as directed.

Do not use product past the expiration date shown on the container.

References

1. Sutter, V. L. and W. T. Cater. 1972. Evaluation of media and reagents in indole-spot test in anaerobic bacteriology.

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Am. J. Clin. Path. 58: 335 - 338.

2. Lombard, G. L. and V. R. Dowell, Jr. 1983. Comparison of three reagents for detecting indole production by anaerobic bacteria in microtest systems. *J. Clin Microbiol.* 18: 609 - 613.

3. Jousimies-Somer, H. R., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. and S. M. Finegold. 2002. *Wadsworth - KTL Anaerobic Bacteriology Manual.* Star Publishing Co., Belmont, CA 94002.

4. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1977. *Anaerobe Laboratory Manual.* Virginia Polytechnic Institute and State University. Blacksburg, VA 24061

5. Isenberg, H. D. 1992. *Clinical Microbiology Procedures Handbook.* American Society for Microbiology Publishing, Washington, D.C. 20005.

6. Murray, R. P., et al. 1999. *Manual of Clinical Microbiology.* American Society for Microbiology Publishing, Washington, D.C. 20005.

Further Information

For further information please contact your local MICROMASTER Representative.



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