# Product Insert

# NITRATE REAGENTS A & B

## Products

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|---|---|---|
| AS-704 | Nitrate Reagents A & B | 1 bottle each / set |

## Intended Use

Nitrate Reagents A & B are used for the Nitrate Reduction Test to determine the ability of an organism to reduce nitrate to nitrite or free nitrogen gas.

## Summary

Nitrate Reagent A & B is used to determine if an organism has the ability of reducing nitrate to nitrite or free nitrogen gas. Organisms that have the enzyme nitrate reductase can reduce nitrate into a usable source of nitrogen. Biochemical tests are valuable for identification of anaerobic bacteria. Utilization of nitrate discs, when placed on conventional media, has been described as a rapid method for the detection of nitrate reduction by anaerobic bacteria.

## Formulation*

| | | |
|---|---|---|
| **Nitrate Reagent A** | | |
| Glacial Acetic Acid | 60.00 | mL |
| Sulfanilic Acid | 1.00 | g |
| DI Water | 240.00 | mL |
| | | |
| **Nitrate Reagent B** | | |
| Glacial Acetic Acid | 60.00 | mL |
| 1,6-Cleve's Acid | 0.40 | g |
| DI Water | 890.00 | mL |

Final volume: 30.0 mL ± 3.0 mL

*Approximate formula. Adjusted and/or supplemented as required to meet performance criteria.

## Precautions

For *IN VITRO DIAGNOSTIC USE* only. 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.

## Storage and Shelf Life

**Storage:** Upon receipt, store at store at room temperature in original packaging until use. Do not use the product if there are signs of deterioration to the package. 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 label.

**Shelf Life:** 1 year from date of manufacture.

## Procedure

**Nitrate Reduction Test:** Inoculate a supportive agar, like Brucella Blood Agar (AS-111), with a pure culture of the organism to be tested. Place a Nitrate Disk (AS-703) in the area with the heaviest inoculum and incubate anaerobically for 24 – 48 hours. Remove the disk from the surface of the plate with sterilized tweezers and place on a clean petri dish or slide. Add 1 drop of each reagent A and B. Observe for a few minutes for a color change. If no color develops, drop a small amount of zinc dust (AS-709) onto the surface of the disk and observe for a color change for up to 5 minutes.

## Materials Required, But Not Provided

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

## Interpretation of Results

A positive reaction is indicated by a red to pink color change within 1 to 2 minutes of adding the reagents, which means that nitrate was reduced to nitrite. If no color changes occur during the first step, negative reactions are confirmed by the addition of zinc dust. If a color change occurs within 5 minutes after adding zinc, it indicates that nitrate has not been reduced by organism. If no color change occurs after 5 minutes after adding zinc, it indicates that the organism reduced nitrate to free nitrogen gas and is considered a positive reaction.

## Limitations

Do not perform a Nitrate Reduction Test and Spot-Indole Test on the same agar plate. Indole reagent can diffuse through the plate and will show a false positive on the nitrate disk. Nitrate reagents A & B 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.

## Quality Control

The following organisms are routinely used for quality control testing at Anaerobe Systems.

| Organism Tested | ATCC # | Expected Reaction |
|---|---|---|
| Bacteroides fragilis | 25285 | Negative |
| Staphylococcus aureus | 25923 | Positive |
| Escherichia coli | 25922 | Positive |
| Propionibacterium acnes | 6919 | Positive |

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

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

| Organism | ATCC# | Expected Reaction |
|---|---|---|
| B. fragilis | 25285 | Negative |
| E. coli | 25922 | Positive |

**Physical Appearance:** Nitrate Reagent A should appear as a transparent yellow liquid within an opaque plastic bottle. Nitrate Reagent B should appear as a transparent light-pink liquid within an opaque plastic bottle


**Anaerobe Systems**
15906 Concord Circle Morgan Hill, CA 95037 408 782 7557 Fax 408 782 3031
http://www.anaerobesystems.com

## References

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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.
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