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**remel**

## **NITRATE REAGENT A**

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### **INTENDED USE**

Remel Nitrate Reagent A is recommended for use in qualitative procedures to determine the ability of an organism to reduce nitrate to nitrite or free nitrogen gas.

### **SUMMARY AND EXPLANATION**

Nitrate serves as the source of nitrogen for many bacteria and fungi, but it must be broken down to be utilized. The ability to reduce nitrate to nitrite is characteristic of the family *Enterobacteriaceae*.<sup>1</sup> Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates, some being capable of further reducing nitrite to nitrogen gas. The end product of reduction depends on the bacterial species.<sup>2</sup> In 1962, Daubner reported that all members of the *Enterobacteriaceae* tested, with the exception of *Erwinia* species, reduced nitrate in eight hours.<sup>3</sup>

### **PRINCIPLE**

The nitrate reduction test is a two-step procedure. Sulfanilic acid (Nitrate Reagent A) is added to acidify the medium and then naphthylamine (Nitrate Reagent B) is added which reacts with nitrites to produce a red color. Some organisms reduce nitrates to free nitrogen or ammonia. If no color develops upon the addition of reagents, the organism has either reduced the nitrate to free nitrogen or it has not attacked the nitrate at all. Nitrate will react with zinc dust to form nitrite. A red color development after the addition of zinc dust demonstrates that nitrates are present, indicating a negative nitrate reduction test. No formation of a red color after the addition of zinc dust indicates that the organism has reduced the nitrate to free nitrogen or ammonia and is considered a positive nitrate test.<sup>4</sup>

### **REAGENTS (CLASSICAL FORMULA)\***

Sulfanilic Acid (CAS 121-57-3) .....8.0 g  
Glacial Acetic Acid (CAS 64-19-7).....286.0 ml  
Demineralized Water (CAS 7732-18-5) .....714.0 ml

\*Adjusted as required to meet performance standards.

### **PRECAUTIONS**

**DANGER! POISON**, may be harmful or fatal if swallowed. **CORROSIVE**, may cause burns or irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to the Material Safety Data Sheet for additional information.

### **STORAGE**

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use.

### **PRODUCT DETERIORATION**

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

### **SPECIMEN COLLECTION, STORAGE, TRANSPORT**

Specimens should be collected and handled following recommended guidelines.<sup>5</sup>

### **MATERIALS REQUIRED BUT NOT SUPPLIED**

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Nitrate Agar (REF R061526), Nitrate Broth (REF R061532), or other suitable supplemental media, (5) Quality control organisms, (6) Nitrate Reagent B (REF R21242), (7) Zinc dust, and (8) Pipettes.

### **PROCEDURE**

**Note:** If using Nitrate Broth and adequate growth is questionable, transfer a portion of incubated broth to a separate tube, reincubate original tube, and add reagents to separate tube.

1. Dispense 5 drops each of Nitrate Reagents A and B into an inoculated Nitrate Broth or Nitrate Agar, which has been incubated at 35-37°C for 24 hours or longer.
2. Observe for the development of a red color within 1-2 minutes after addition of reagents indicating a positive, completed test.
3. If a negative test results (no red color), add a pinch of zinc dust to the medium.
4. Observe for the development of a red color within 5-10 minutes.

## INTERPRETATION

- Positive Test - Red color development within 1-2 minutes after addition of Nitrate Reagents A and B or no red color development within 5-10 minutes after addition of zinc dust.
- Negative Test - No red color development within 1-2 minutes after addition of Nitrate Reagents A and B and red color development within 5-10 minutes after addition of zinc dust.

## QUALITY CONTROL

All lot numbers of Nitrate Reagent A have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Escherichia coli</i> ATCC® 25922	Aerobic, 18-24 h @ 35-37°C	Positive
<i>Acinetobacter baumannii</i> ATCC® 19606	Aerobic, 18-24 h @ 35-37°C	Negative

## LIMITATIONS

1. Color reactions with a positive test may fade quickly; interpretations should be made soon after addition of reagents.<sup>2</sup>
2. A strong nitrate-reducing organism may exhibit a brown precipitate immediately after the addition of the reagents.<sup>2</sup>
3. When adding zinc, do not use an excess; if too much zinc is added, the large amount of hydrogen gas produced may reduce the nitrite (formed from unreduced nitrate) to ammonia (NH<sub>3</sub>), which could give a false-negative result (no color) or just a fleeting color reaction.<sup>2</sup>




## BIBLIOGRAPHY

1. Ewing, W.H. 1986. Edwards & Ewing's Identification of Enterobacteriaceae. 4<sup>th</sup> ed. Elsevier, New York, NY.
2. MacFaddin, J.F. 2000. Biochemical Tests For The Identification of Medical Bacteria. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia, PA.
3. Daubner, I. 1962. Arch. Hyg. Berlin. 146:147-150.
4. Hopkins, J.M. and G.A. Land. 1977. J. Clin. Microbiol. 5:497-500.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover. 2003. Manual of Clinical Microbiology. 8<sup>th</sup> ed. ASM, Washington, D.C.
6. Wallace, G.I. and S.L. Neave. 1927. J. Bacteriol. 14:377-384.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's Diagnostic Microbiology. 11<sup>th</sup> ed. Mosby, St. Louis, MO.

## PACKAGING

REF R21239, Nitrate Reagent A ..... 25 ml/Btl

## Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

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