NITRATE REAGENTS

- For in vitro use only - Cat No. RN75, RN76 & RZ75

Our Nitrate Reagents are used to detect an organism's ability to reduce nitrate to nitrite.

Many bacteria possess the enzymes needed to reduce nitrate. The reduction of nitrate provides the organisms with oxygen that serves as the final electron acceptor in the respiration process used to generate energy. Bacterial reduction of nitrate results in the production of various end products that commonly include nitrite, ammonia, nitrogen gas, nitric oxide, nitrous oxide, and hydroxylamine. The final end product observed depends on the bacterial species and the environmental conditions. The nitrate reduction characteristic of a particular species is more or less constant making it a useful diagnostic tool.

Common nitrate reduction tests include various types of nitrate broths, agars, as well as nitrate disks (anaerobes). If nitrate was reduced to nitrite then reagent A, a 0.8% sulfanilic acid solution, reacts with nitrite to form a diazonium salt. The resultant diazonium salt reacts with Reagent B, a 0.6% N,N-dimethyl-1-naphthylamine solution, to form a red, water soluble azo dye. Therefore, a red color change signals that nitrate has been reduced to nitrite, a positive nitrate reduction test. If no color change is detected, zinc dust must be added before a final interpretation can be made. Zinc Dust (Dalynn RZ75) is a reducing reagent that can rapidly reduce nitrate to nitrite. Therefore a red color change after the addition of zinc dust signifies that nitrate is still present and was not reduced by the bacteria, a negative result. However, if no color change occurs, then this indicates that nitrate is not present and has been reduced beyond nitrite to ammonia or nitrogen gas, a positive test for nitrate reduction.

Formulation

RN75-25 Nitrate Reagent A (25-mL)

Sulfanilic Acid	0.2 g
Acetic Acid (5N)	25.0 mL

RN76-25 Nitrate Reagent B (25-mL)

N,N-Dimethyl-1-naphthylamine	0.15 g
Acetic Acid (5N)	25.0 mL

RZ75-05 Zinc Dust (5-g)

Zinc Powder	5.0 g

Recommended Procedure

Please refer to the appropriate technical sheet depending on the nitrate medium used for a detailed testing procedure.

Interpretation of Results

Positive Test for Nitrate Reduction

Nitrate reduced to nitrite:

Pinkish-red color change after addition of Nitrate Reagents A & B

Nitrate reduced beyond nitrite:

No color change after addition of Nitrate Reagents A & B and no color change after addition of Zinc Dust

Negative Test for Nitrate Reduction

Nitrate not reduced:

No color change after addition of Nitrate Reagents A & B and pinkish-red color change after addition of Zinc Dust

- *In some instances nitrate may only be partially reduced or an organism may temporarily lose its ability to reduce nitrate*
- *Do not add an excessive amount of zinc dust as the large amount of hydrogen gas produced may reduce the nitrate beyond nitrate to ammonia resulting in erroneous findings*

Quality Control

To ensure performance of the reagents, the following control strains are inoculated into a nitrate broth and incubated for 24 hours at 35°C. 5 drops of Nitrate Reagent A & 5 drops of Nitrate Reagent B are added to the overnight culture and the reactions are read after 2 minutes. If needed, 20 mg of zinc dust is added to the tube.

Organism	Expected Results	
Escherichia coli ATCC 25922	+ve	Pink to red after addition of reagents
Acinetobacter lwoffii ATCC 15309	-ve	No colour change after addition of reagents, red colour on addition of zinc

Storage and Shelf Life

Our Nitrate Reagents should be stored at 4°C to 8°C. At this temperature they have a shelf life of 52 weeks from the date of manufacture.

References

1. Cowan ST. Cowan & Steel's manual for the identification of medical bacteria. 2nd ed. Cambridge: Cambridge University Press, 1974.
2. American Public Health Association, Committee on Laboratory Standards. Bacterial nitrate reduction test: suggestions for use of alternate (noncarcinogenic) reagents. Am Soc Microbial News 1975; 41:225-7.
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
4. Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, Eds. Manual of clinical microbiology. 5th ed. Washington, DC: ASM, 1991.
5. MacFaddin JF. Biochemical tests for the identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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