Nitrate Reduction Reagent (Zinc Reducing Agent)

N1508557

Storage: Room Temperature. Store in the dark.

Shipping: Normal.

Introduction:

Microbial respiration is primarily divided into aerobic respiration and anaerobic respiration. The oxidase test, catalase test, and nitrate reduction test are key steps in aerobic or anaerobic respiration and play important roles in bacterial identification.

The nitrate reduction reaction includes two processes: First, in the synthesis process, nitrate is reduced to nitrite and ammonia, and ammonia is subsequently converted into amino acids and other intracellular nitrogen-containing compounds. Second, in the catabolic process, nitrate or nitrite substitutes oxygen as the terminal hydrogen acceptor in the respiratory enzyme system. Bacteria capable of reducing nitrate obtain oxygen from nitrate to form nitrite and other reductive products. The nitrate reduction process varies among bacteria. Some bacteria only reduce nitrate to nitrite, such as Escherichia coli; some can reduce it to nitrite and ammonium ions; some bacteria can reduce nitrate or nitrite to nitrogen, such as Pseudomonas and Serratia species; others may fully utilize the reduction products in anabolic metabolism.

Nitrate Reduction Reagent (Zinc Reducing Agent) primarily consists of zinc powder. It can be used in conjunction with nitrate reduction test reagents and is commonly employed in nitrate reduction tests (Griess test) for the reduction of nitrate. It is mainly used for the identification of Enterobacteriaceae, Pseudomonas, etc. Bacteria such as Escherichia coli only reduce nitrate to nitrite. Under acetic acid conditions, nitrite reacts with sulfanilic acid in the reagent to form diazobenzenesulfonic acid, which then combines with α-naphthylamine to produce a red product. Bacteria such as Pseudomonas and Serratia can reduce nitrate or nitrite to nitrogen or nitrogen oxides, a process known as denitrification or denitrogenation, which produces bubbles but no red color appears.

This reagent is intended for research use only and is not suitable for clinical diagnosis or other purposes.

Reagents, consumables and Equipments not provided:

- Nitrate medium, Nitrate reduction test reagent.
- Constant temperature incubator, Test tubes, Alcohol burner.
- Inoculation loop, Inoculation hood, Precision balance (accuracy 0.1 mg).

Procedure (For Reference Only):

1. Inoculate the test bacteria into nitrate medium and incubate at 35°C for 1–4 days.

2. Mix equal volumes of sulfanilic acid solution and α-naphthylamine solution to prepare the nitrate reduction test reagent.
3. Add 0.1 mL of the nitrate reduction test reagent to the culture medium and observe the result immediately.
4. If no red color appears after adding the nitrate reduction test reagent, add a small amount of zinc reducer (1–3 mg) to the test tube. If red color develops, it indicates that nitrate is still present in the medium. If no red color appears, it indicates that nitrate has been reduced to ammonia or nitrogen. A small inverted tube can be placed in the medium; if bubbles are generated, it indicates nitrogen gas production, helping to rule out false negatives.

Result Interpretation:

Condition	Color Result	Interpretation (Example Organisms)
Without zinc reducer	Red	Positive (e.g., Enterobacteriaceae, *Veillonella*)
With zinc reducer	Red	Negative
With zinc reducer	No color change	False negative (e.g., *Pseudomonas*, *Serratia*)

Note: If no color reaction occurs after adding the reagent, possible reasons are: 1. Nitrate was not reduced – true negative; 2. Nitrate was reduced to ammonia, nitrogen, or other substances, leading to a false negative. In this case, add a small amount of zinc reducer. If red color appears, the test is truly negative; if no red color appears, the test is a false negative.

Precautions:

1. Take appropriate self-protection measures during bacterial culture and experimental procedures.
2. The incubation time of the test bacteria can also affect staining. Over-incubation, death, or lysis of bacteria often leads to negative reactions.
3. Some anaerobic bacteria (e.g., Veillonella) may also show positive reactions.
4. Results must be judged immediately after adding the reagent; otherwise, rapid fading of the color may make interpretation difficult, as with Pseudomonas aeruginosa and Stenotrophomonas maltophilia
5. Sulfanilic acid solution and α-naphthylamine solution contain acetic acid and have an irritating odor; avoid inhalation.
6. Zinc reducer is potentially hazardous; handle with caution. Avoid contact with water, store protected from light and in a ventilated area.
7. For your safety and health, please wear a lab coat and disposable gloves during operation.