73426 Nitrate Reduction Test

Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrite or nitrogenous gases. The reduction of nitrate may be coupled to anaerobic respiration in some species.

Kit content	Qty.
1. Nitrate Broth (72548) *Peptone 5 g/l* *Meat extract 3 g/l* *Potassium nitrate 1 g/l* *Final pH 7.0 ± 0.2 at 25°C*	100 g
2. Sulfanilic acid (86090) = Reagent A	100 g
3. N,N-Dimethyl-1-naphthylamine (D4011) = Reagent B	10 ml
4. Zinc (93027)	1 g

(all kit components are available as single components)

Preparation of Reagents:
Sulfanilic acid solution (Reagent A): Dissolve 8 g of sulfanilic acid in 1 litre 5N acetic acid. Store Reagent A at room temperature for up to 3 months, in dark. Reagents may be stored in dark brown glass containers; bottles may be wrapped in aluminum foil to ensure darkness.

α-Naphthylamine solution (Reagent B): Dissolve 6 g of N,N-Dimethyl-1-naphthylamine in 1 litre 5N acetic acid. Store Reagent B at 2 to 8°C for up to 3 months, in dark. Reagents may be stored in dark brown glass containers; bottles may be wrapped in aluminum foil to ensure darkness.

Directions:
Dissolve 9 g of Nitrate Broth in 1 litre distilled water. Dispense 10 ml aliquots of the broth into tubes fitted with Durham tubes. Sterilize by autoclaving at 121°C for 15 minutes.

Inoculate the tubes heavily with a fresh culture of the suspect organism. Inoculate at least 1 ml sample in a tube or take a big part of a colony with an inoculating loop. Do not forget a negative control without any bacteria.

A successful nitrate reduction test is dependent on performing the test under the correct conditions. That means the organisms needs the accurate growth media, the correct temperature and anaerobic conditions. The nitrate broth from this kit can be used for the most usual bacteria like Enterobacteriaceae, Bacilli, Salmonella and others. For special organisms the media maybe has to be modified or Sigma-Aldrich provides other media e.g. the Motility Nitrate Agar (14305). Nitrate reaction occurs only under anaerobic conditions. The medium is dispensed in tubes to give a low surface area to depth ratio which limits the diffusion of oxygen into the medium. Most bacteria use the oxygen in the medium and rapidly produce anaerobic conditions. To reach faster an anaerobic condition it is recommendable to give about one centimetre of paraffin oil on the surface of the media or over-gassing with e.g. carbon dioxide and seal the tube with parafilm.

Incubate the tubes at 35 to 37°C (bacilli at 30°C) for 24 to 48 h in an incubator with or without supplemental carbon dioxide.

Put a 5 drops of reagent A and 5 drops of reagent B into the tube containing culture to be tested. Shake the tube well to mix reagents with medium. A distinct red or pink colour, which should develop within a few minutes, indicates nitrate reduction.

If the suspension turns pink-red before the addition of Zn powder, the reaction is positive and the test is completed.
If the suspension is colorless after the addition of reagents A and B, add a small amount ("sharp knife point") of zinc powder to the medium. Shake the tube vigorously and allow it to stand at room temperature for 10-15 min.
If the medium remains colorless after the addition of Zn powder, the test result is positive.
If the medium turns pink after the addition of Zn powder, the result is negative.
The negative control should also be tested. There should be no pink colour formation after adding reagent A and B and if zinc powder is added the colour should change to pink. Nitrate reduction is not a confirmatory test. Complete identification should include the morphology, gram reaction, biochemical and serological tests. Addition of too much zinc powder can results in false-negative reaction.

Principle and Interpretation:
Nitrate, present in the broth, is reduced to nitrite which may then be reduced to nitric oxide, nitrous oxide, or nitrogen (see Figure 1). The nitrate reduction test is based on the detection of nitrite and its ability to form a red compound when it reacts with sulfanilic acid (reagent A) to form a complex (nitrite-sulfanilic acid) which then reacts with a α-naphthylamine (Reagent B) to give a red precipitate (prontosil) as shown in Figure 1. Zinc powder catalyses the reduction of nitrate to nitrite.

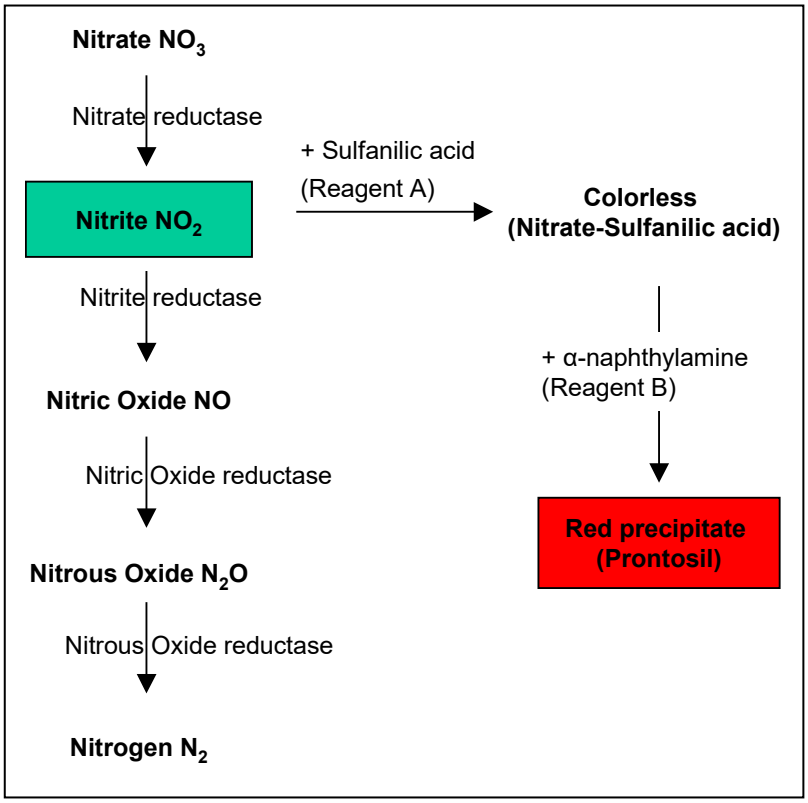

Figure 1: Biochemical nitrate reduction pathway and detection of nitrite in medium

Reduction of nitrate is generally an anaerobic respiration in which an organism derives its oxygen from nitrate.

A red color will be produced in the medium only when nitrite is present in the medium. Lack of a red color in the medium after the addition of sulfanilic acid and α-naphthylamine means only that nitrite is not present in the medium. There may be two explanations for this observation.

- The nitrate may not have been reduced; the strain is nitrate-negative.
- The nitrate may have been reduced to nitrite which has then been completely reduced to nitric oxide, nitrous oxide, or nitrogen which will not react with the reagents that react with nitrite; the strain is nitrate-positive.

Cultural characteristics after 24-48 hours at 35 to 37°C.

Organisms (ATCC)	Growth	Nitrate reduction
Acinetobacter calcoaceticus (19606)	+++	-
Enterobacter aerogenes (13048)	+++	+
Escherichia coli (25922)	+++	+
Salmonella typhimurium (14028)	+++	+

References:

1. Society of American Bacteriologist, Pure Culture Study of Bacteria, 12 : Leaflet 11, 8 (1944)
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3. International Organisation for Sandardization (ISO), Draft ISO/DIS 7932 (1993)
4. J.F. Mac Faddin, Biochemical Tests for the identification of Medical Bacteria, 2nd ed., Baltimore, MD.: Williams & Wilkins (1980)
5. J.S. Knapp, V.L. Clark, Anaerobic growth of Neisseria gonorrhoeae coupled to nitrite reduction, Infect. Immun. 46,176-181 (1984)
6. V.B.D. Skerman, A guide to the identification of the genera of bacteria, The Williams & Wilkins Co., Baltimore, MD, p.218 - 220 (1967)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.