72548 Nitrate Broth

Recommended for detecting nitrate-reducing and indole-producing microorganisms. Recommended by the "Schweizerisches Lebensmittelbuch" 5th ed., chapter 56A.

Composition:

Ingredients	Grams/Litre
Peptone	5.0
Meat extract	3.0
Potassium nitrate	1.0
Final pH 7.0 ± 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Dissolve 9 g in 1 litre distilled water. Dispense in tubes and 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.

Indole test: See Kovac's Reagent for indoles (67309)

Nitrate test:
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. This nitrate broth can be used for the most usual bacteria like Enterobacteriaceae, Bacilli, Salmonella and others. For special organisms the media maybe has to be modified. 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 may 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.
Addition of too much zinc powder can results in false-negative reaction.

Preparation of Reagents:
Sulfanilic acid solution (Reagent A): Dissolve 8 g of sulfanilic acid (86090) 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 (40860) 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.

Principle and Interpretation:
Nitrate media are prepared in accordance with the formula published in "Pure Culture Study of Bacteria" of the Society of American Bacteriologist (1). The ability to reduce nitrate is valuable for differentiating and identifying various type of bacteria especially Enterobacteriacea family (2). Recently ISO committee (3) has recommended Nitrate Broth for the enumeration of Bacillus cereus – colony count technique at 30°C.
Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable to denitrify (reduction of nitrate to nitrogen). For the glucose fermenting gram-negative bacilli, the production of nitrogen gas from nitrate is an important differential test (4). Reduction of nitrate is generally an anaerobic respiration in which organismderives its oxygen from nitrate. Members of Enterobacteriacea characteristically reduce nitrate to nitrite which reacts with sulfanilic acid and N,N-Dimethyl-1-naphthylamine to produce the red colour. This reaction is also known as Griess reaction.
Nitrate reduction is not a confirmatory test. Complete identification should include the morphology, gram reaction, biochemical and serological tests.

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)
2. Ewing, Edwards and Ewings Identification of Enterobacteriaceae, 4th ed., Elsevier Science Pub. Co., Inc., N.Y. (1986)
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.