

Nitrate Broth (i23220)

Recommended as an aid in the identification of aerobic and facultative anaerobic gram-negative microorganisms by means of the nitrate reduction test.

Industry: Food

Principles & Uses

Nitrate Broth is essential for differentiating bacteria, particularly in the *Enterobacteriaceae* family, based on their nitrate reduction capabilities. Non-fermenters and gram-negative bacilli exhibit varying nitrate reduction abilities, with some capable of denitrification, reducing nitrate to nitrogen gas. For glucose-fermenting gram-negative bacilli, the production of nitrogen gas from nitrate serves as a crucial differential test. The medium includes potassium nitrate as a substrate, with certain bacteria converting nitrate to nitrite, ammonia, or nitrogen gas.

The Griess reaction is employed for nitrate detection, involving the addition of sulphanic acid and alpha-naphthylamine solutions. A red or pink color indicates nitrate reduction, with further verification possible by adding zinc dust, which results in a red color if nitrate is still present. Nitrate reduction, though not confirmatory, provides insight into bacterial metabolism. Members of the *Enterobacteriaceae* family typically reduce nitrate to nitrite, producing a distinct red color in positive reactions. Excess zinc can lead to false negatives, and during the nitrate reduction test with alpha-naphthylamine, color fading in positive reactions is possible.

Composition (gr/L)

Peptone 5, Beef Extract 3, Potassium Nitrate 1.

Final pH at 25°C 7.0 ± 0.2

Preparation from dehydrated Powder

Suspend 9 g of the medium in 1 L of distilled water and mix well. Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures.

Preparation of Reagents

Reagent A, a sulfanilic acid solution, is prepared by dissolving 8 g of sulfanilic acid in 1 liter of 5N acetic acid. This solution is stored at room temperature for up to 3 months in darkness, using dark glass containers or aluminum foil-wrapped bottles. Reagent B, an α -Naphthylamine solution, is created by dissolving 6 g of N,N-Dimethyl-1-naphthylamine in 1 liter of 5N acetic acid. It is stored in darkness at 2 to 8°C for up to 3 months using similar light-protective measures.

Quality Control

Dehydrated Appearance: Light to medium tan, free-flowing, homogeneous.

Prepared Appearance: Light to medium amber, clear.

Reaction of 0.9 % Solution at 25°C: pH 7.0 ± 0.2

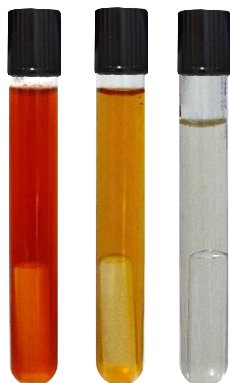
Cultural Response

Inoculate and incubate at 35 ± 2°C for 18-24 hours. Test for nitrate reduction using Nitrate A Reagent, Nitrate B Reagent and Nitrate C Reagent.

Note: If the suspension is colorless after the addition of reagent A and B. add a small amount of zinc to the medium. if the medium remains colorless after the addition zinc the test result is positive if the medium turns pink after the addition of zinc powder the result is negative.

Organism (ATCC*)	Recovery	Nitrate Reduction
<i>Acinetobacter calcoaceticus</i> (19606)	Good	-
<i>Enterobacter aerogenes</i> (13048)	Good	+
<i>Escherichia coli</i> (25922)	Good	+
<i>Pseudomonas aeruginosa</i> (27853)	Good	+
<i>Salmonella enterica</i> (14028)	Good	+

*ATCC is a registered trade mark of the American Type Culture Collection.



Enterobacter aerogenes with positive Nitrate reduction (left).
Streptococcus agalactiae with negative Nitrate Reduction (middle). Prepared Culture Medium (right).

Storage

Keep the container at 15-30 °C. Store prepared medium at 2-8 °C.