NITRATE BROTH

Medium for aerobic and facultative anaerobic gram-negative bacteria differentiation by means of the nitrate reduction test.

TYPICAL FORMULA (g/L)

Peptone	5.0
Meat Extract	3.0
Potassium Nitrate	1.0
Final pH 7.0 ± 0.2	

DESCRIPTION

NITRATE BROTH is a medium for the identification of aerobic and facultative anaerobic gram-negative microorganisms by means of the nitrate reduction test.

PRINCIPLE

The nitrate reduction to nitrite is a metabolic capability of microorganisms that subtract oxigen from nitrate to form nitrite. The nitrites are colourless and react with the reagents sulphanilic acid and alpha-naphthylamine with the formation of a red compound. In the positive reaction, microrganisms reduce nitrates to nitrites and after the addition of the two reagents in the culture medium, a red-orange colour develops. In the negative reaction, microorganisms are unable to reduce nitrates to nitrites and so no colour develops in the culture medium after the addition of the two reagents. In case of negative reaction - no red orange colour development- it is necessary to add a small quantity of zinc powder: metallic zinc reduces nitrates to nitrites. The red-orange colour development, after its addition, indicates that nitrates were present initially and they were reduced to nitrites by metallic zinc and not by bacteria (nitrates reduction to nitrites: negative); the absence of a red-orange colouring, after the addition of zinc, indicates that nitrates have been reduced at first to nitrites and subsequentially to other compounds (nitrates reduction to nitrites: positive).

PREPARATION

1. Dissolve 9 g of the powder in 1 L of purified water.
2. Heat to boiling and mix until completely dissolved.
3. Distribute into final containers.
4. Autoclave at 121 °C for 15 minutes.

TECHNIQUE

Inoculate the medium with a colony that is well isolated on the isolation medium. Replace cap loosely and incubate at 35 ± 2 °C for 24 hours.
Examine the tubes after 18-24 hours for growth.
Add 10 drops of alpha-naphthylamine reagent and 10 drops of sulphanilic acid reagent and await some seconds. Observe the development of a red-orange colouring. In case of negative reaction add a small quantity of zinc powder and observe the eventual development of the red-orange colouring.
Nitrate reduction is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, biochemical and serological tests.

STORAGE

10-30°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

WARNING and PRECAUTIONS

The product is not classified as hazardous by current legislation and does not contain harmful substances in concentrations of ≥1%.
The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL of WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. *Ewing 1986. Edwards & Ewing's. Identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co. Inc. New York.*
2. *MacFaddin 1980. Biochemical Tests for the identification of medical bacteria, 2nd ed. Williams & Wilkins, Baltimore.*
3. *Finegold and Baron. 1986 Bailey and Scott's. Diagnostic microbiology, 7th ed. The C.V. Mosby Company, St. Louis.*
4. *Kelly, Brenner and Farmer. 1985. In Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. ASM, Washington, D.C.*

Liofilchem s.r.l.
Via Scozia, Zona industriale - 64026 Roseto degli Abruzzi (Te) Italy - Tel. +39.0858930745 - Fax +39.0858930330
Web site: http://www.liofilchem.net - E-mail: liofilchem@liofilchem.net

PRODUCT SPECIFICATIONS

NAME
NITRATE BROTH

PRESENTATION
Dehydrated powder.

STORAGE
10-30 °C

PACKAGING

Code	Content	Packaging
610322	500 g	500 g of powder in plastic bottle
620322	100 g	100 g of powder in plastic bottle

pH OF THE MEDIUM
7.0 ± 0.2

USE
NITRATE BROTH is a medium for the identification of aerobic and facultative anaerobic gram-negative microorganisms by means of the nitrate reduction test.

TECHNIQUE
Refer to technical sheet of the product.

APPEARANCE of the MEDIUM

Dehydrated medium
Appearance: free-flowing, homogeneous.
Colour: Light to medium tan
Prepared medium
Appearance: clear
Colour: light to medium amber

SHELFLIFE
4 years

QUALITY CONTROL

1. Control of general characteristics, label and print
2. Sterility control
   7 days at 25 ± 1°C, in aerobiosis
   7 days at 36 ± 1°C, in aerobiosis
3. Microbiological control

Inoculum for productivity: 10-100 UFC/ml
Inoculum for selectivity: 10^4-10^5 UFC/ml
Inoculum for specificity: ≤ 10^4 UFC/ml
Incubation conditions: 24 h at 35 ± 2 °C

Microorganism		Growth	Nitrate reduction
Acinetobacter calcoaceticus	ATCC 19606	Good	-
Enterobacter aerogenes	ATCC 13048	Good	+
Pseudomonas aeruginosa	ATCC 27853	Good	+
Escherichia coli	ATCC 25922	Good	+

TABLE OF SYMBOLS

LOT Batch code	Temperature limitation	Manufacturer	Contains sufficient for <n> tests	IVD *In vitro* Diagnostic Medical Device
REF Catalogue number	Keep away from heat	Use by	Caution, consult accompanying documents	

CE IVD

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