

TECHNICAL DATA SHEET

Nitrate Agar

Principle

Nitrate agar is composed of peptone, meat extract (equivalent of beef extract), sodium nitrate, and agar. Peptone and meat extract provides nitrogen, amino acids, carbon and other essential nutrients. Potassium nitrate is a source of nitrate and agar is solidifying agent. The nitrate reducing bacteria converts nitrate to nitrites. The produced nitrites are detected by adding few drops (about 0.5 ml) of 0.5% α -naphthylamine (Nitrate reagent A) and 0.8% sulphanilic acid (Nitrate reagent B). In acidic environment, the nitrites react with alpha-naphthylamine to produce a pink or red dye i.e. p-sulfobenzene-azo-a-naphthylamine, indicates nitrate reduction to nitrite. If there is no color change after adding the reagents, add a small amount of zinc powder to the broth. If the broth turns red after adding zinc powder, it indicates that nitrate was not reduced to nitrite. If there is still no color change, it suggests that nitrate was reduced to a nitrogenous compound other than nitrite.

Use: For detection of nitrate reducing bacteria.

Contents*

Ingredients	Gram/Liter
Peptone	5.000
Meat Extract#	3.000
Potassium Nitrate	1.000
Agar	12.000
pH at 25°C	6.8±0.2

*** Formula adjusted for optimum performance and parameters**

Equivalent to Beef Extract

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Directions: Dissolve 21 grams in 1000 ml distilled water, boil to dissolve the medium completely and distribute into test tube. Sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 min, cool it to 42-45°C and place the tube for preparation of slant. Inoculate test sample aseptically. After incubation of 24 hours add few drops of Nitrate reagent A and B. Observe for development of a distinct red or pink color within 1-2 minutes, indicating reduction of nitrate to nitrite.

Preparation of nitrate reagent A and B

Nitrate reagent A: 0.5 g α -naphthylamine (a carcinogen) in 100 ml 5 N acetic acid by gently heating. Nitrate reagent B: 0.8 g sulfanilic acid in 100 ml 5 N acetic acid by gently heating.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Light Beige colored free flowing, homogeneous powder
Reaction of 2.1% solution	6.8±0.2 at 25°C
pH	6.60 – 7.00
Gelling	Firm comparable with 1.2% agar gel
Color and clarity of ready medium	Light amber colored opalescent gel
Growth Promotion properties	Best at ≤ 100 CFU at 32-37 °C for 18-48 h
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 18-24 h
Negative control	Performed using sterile distilled water

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Different Microbial Response Cultural characteristics observed after incubation at $35\pm 2^{\circ}\text{C}$ for 18-24 hours. Nitrate reduction observed after adding 0.5ml of each Nitrate reagent A and B.

Organism	ATCC	Inoculum (CFU)	Growth	Nitrate reduction
<i>Escherichia coli</i>	8739	50-100	Luxuriant	Positive (red color developed)
<i>Salmonella typhimurium</i>	14028	50-100	Luxuriant	Positive (red color developed)
<i>Klebsiella aerogenes</i>	13048	50-100	Luxuriant	Positive (red color developed)
<i>Streptococcus pneumoniae</i>	6303	50-100	Luxuriant	Negative (no red color developed)

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998.
2. Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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