

TECHNICAL DATA SHEET

Nitrate Broth

Principle

Nitrate broth is composed of peptone, meat extract (equivalent of beef extract) and sodium nitrate. Peptone and meat extract provides nitrogen, amino acids, carbon and other essential nutrients. Potassium nitrate is a source of nitrate. The nitrate reducing bacteria converts nitrate to nitrites. The produced nitrites are detected by adding α -naphthylamine. In acidic environment, the nitrites react with alpha-naphthylamine to produce a pink or red color. While, nitrate negative organisms, unable to reduce nitrates, and yield no color after the addition of α -naphthylamine. Nitrate-negative reactions are tested with zinc dust to confirm the presence of unreduced nitrate.

Use: For detection of nitrate reduction and enumeration of *Bacillus cereus*.

Contents*

Ingredients	Gram/Liter
Peptone	5.00
Meat Extract#	3.00
Potassium Nitrate	1.00
pH at 25°C	7.0 $\pm$0.2

* Formula adjusted for optimum performance and parameters

Equivalent to Beef Extract

Directions: Dissolve 9.0 grams in 1000 ml distilled water, boil to dissolve the medium completely and distribute aseptically. Sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 min, cool it to 42-45 °C and inoculate test sample aseptically. After incubation of 24 hrs. add few drops of 0.8% sulfanilic acid and α -naphthylamine (0.5 g in 100 ml DW) to each tube. Observe for development of a distinct red or pink color within 1-2 minutes, indicating reduction of nitrate to nitrite.

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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 0.9% solution	7.0 $\pm$ 0.2 at 25 °C
pH	6.80- 7.20
Color and clarity of ready medium	Light amber colored solution
Growth Promotion properties	Best at $\leq$ 100 CFU at 32-37 °C for 18-72 h
Indicative properties	Optimum at $\leq$ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

Different Microbial Response Cultural characteristics observed after incubation at 33-37°C for 18-24 hours.

Organism	ATCC	Inoculum	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)

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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. Atlas, R. M. (2005). *Handbook of media for environmental microbiology*. CRC press.
2. *Difco Manual* (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.

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