



Nutrient Broth, pH 6.9 w/o NaCl

M088

Intended use

Recommended as general purpose medium for the cultivation of microorganisms.

Composition**

Ingredients	g/ L
Peptone	5.000
HM Peptone B [#]	3.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

[#] - Equivalent to Beef extract

Directions

Suspend 8.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Nutrient Broth with pH 6.9 and devoid of NaCl is a relatively simple formulation containing HM Peptone B and peptone which can support the growth of non-fastidious microorganisms. It provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. It has the formula originally developed for use in Standard Methods for the Examination of Water and Waste Water (1). It is recommended by FDA BAM (2) and APHA (3) for enrichment of Frosting and topping mixes samples for detecting presence of *Salmonella*.

If this medium is incorporated with 6.5% sodium chloride, then it acts as a differential and selective agent by interfering with the osmotic balance and the permeability of membrane of majority of microorganisms. On addition of 6.5% Sodium chloride, the medium can also be used to determine salt tolerance of bile esculin positive Enterococci (4).

Type of specimen

Water samples, Food samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).

For food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2,3).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Further isolation and biochemical testing must be performed for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder.

Colour and Clarity of prepared medium

Light amber coloured clear solution without any precipitate

Reaction

Reaction of 0.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	good-luxuriant
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50-100	good-luxuriant
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant
<i>Salmonella Typhi</i> ATCC 6539	50-100	good-luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	good-luxuriant
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	good-luxuriant

Key : *Corresponding WDCM numbers

Formerly known as *Enterobacter aerogenes*.**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
2. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
3. Wallace H. Andrews (ret.), Hua Wang (2022), BAM Chapter 5: Salmonella.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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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