



Halophilic Agar

Intended Use:

Recommended for isolation and cultivation of extremely halophilic bacteria.

Composition**

Ingredients	Gms / Litre
Acicase™ #	10.000
Yeast extract	10.000
Proteose peptone	5.000
Sodium citrate	3.000
Potassium chloride	2.000
Magnesium sulphate	25.000
Sodium chloride	250.000
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Casein acid hydrolysate

Directions

Suspend 32.5 grams in 100 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Halophilic media are formulated for isolation and cultivation of extreme halophilic species of *Halobacterium* and *Halococcus* from foods (1, 2). For optimum growth they require high salt concentration of about 20-30%. In general, the requirement for salt by halophilic microorganisms is not an exclusive need for NaCl since many species require low levels of K⁺, Mg⁺⁺ and other cations anions in addition to NaCl (5,6). These bacteria can cause pink discoloration on the outer surface accompanied by putrefaction and decomposition of fish, bacon and hides preserved in sea salts. Halophilic Agar contains Acicase™, proteose peptone and yeast extract which provide all the necessary nutrients, mainly nitrogenous, carbonaceous compounds, long chain amino acids. and vitamins to the halophilic bacteria. Sodium citrate is added to avoid the losses (2). Magnesium sulphate, sodium chloride and potassium chloride are essential ions required for the growth of extreme halophiles.

Type of specimen

Water samples from high-salinity water body

Specimen Collection and Handling:

10 gm sample is added to 90 ml Halophilic Broth (M591) and incubated at 35°C for upto 12 days. The organisms are then isolated onto Halophilic Agar (M590) from this enriched culture.

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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

Off-white to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Amber coloured, slightly opalescent gel w/ precipitate forms in Petri plates.

Reaction

Reaction of 32.5% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 12 days.

Organism**Growth**

Halobacterium salinarium luxuriant

ATCC 33171

Halococcus morrhuae ATCC luxuriant

17082

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

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2. Gibbons N.E., 1969, Methods In Microbiology, Vol. 3B, Norris J.R., and Ribbons D.W. (Eds.), A.P., New York, pp.169-183.
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.
5. Kushner D. J., (Eds.), 1978, D. J. Kushner, pg 317, Academic Press, London, England.
6. MacLeod R. A., 1965, Bacteriol., Rev., 29:9

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