

**Halophilic Agar****M590**

Halophilic Agar is used for the isolation and cultivation of extremely halophilic bacteria.

**Composition\*\*\***

Ingredients	Gms / Litre
Casein acid hydrolysate	10.000
Yeast extract	10.000
Proteose peptone	5.000
Trisodium 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

**Directions**

Suspend 32.5 grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes

**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<sup>+</sup>, Mg<sup>++</sup> and other cations anions in addition to NaCl (3, 4). 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 casein acid hydrolysate, proteose peptone and yeast extract which provide all the necessary nutrients, mainly nitrogenous and vitamins to the halophilic bacteria. Trisodium 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.

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 from this enriched culture.

**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 forms in Petri plates.

**Reaction**

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

**Cultural Response**

M590: Cultural characteristics observed after an incubation at 35-37°C for 12 days.

Organism	Growth					
<i>Halobacterium salinarum</i> ATCC 33171	luxuriant					
<i>Halococcus morrhuae</i> ATCC 17082	luxuriant					

**Reference**

1. Dundas I.E., 1977, Advances In Microbiology and Physiology, Rose H. and Tempest D.W. (Eds.), A.P. London.
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. Kushner D. J., (Eds.), 1978, D. J. Kushner, pg 317, Academic Press, London, England
4. MacLeod R. A., 1965, Bacteriol., Rev., 29:9

**Storage and Shelf Life**

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.