

590. DICHOTOMICROBIUM THERMOHALOPHILUM MEDIUM

Bacto Yeast extract (Difco)	1.000	g
Na ₂ -DL-malate	1.000	g
Hutner's basal salts	20.000	ml
Artificial sea water, 3 times conc.	980.000	ml

pH 7.0-7.2

Agar at a concentration of 18 g/l is added for solid media.

Prepare an 8% solution of NaHCO₃ in half full 100ml serum bottles closed with a rubber septum and autoclave. Add 28.4 ml of the sterile 8% solution of NaHCO₃ (2.822g/l) per litre of the sterile, cooled medium.

Artificial sea water, 3 times concentrated:

NaCl	70.430	g
Na ₂ SO ₄	11.750	g
KCl	1.990	g
KBr	0.290	g
MgCl ₂ x 6 H ₂ O	31.860	g
CaCl ₂ x 2 H ₂ O	4.350	g
H ₃ BO ₃	0.080	g
Distilled water	1000.000	ml

Hutner's salts:

Nitritotriacetic acid (NTA)	10.000	g
MgSO ₄ x 7 H ₂ O	29.700	g
CaCl ₂ x 2 H ₂ O	3.335	g
(NH ₄) ₆ MoO ₇ O ₂₄ x 4 H ₂ O	9.250	mg
FeSO ₄ x 7 H ₂ O	99.000	mg
"Metals 44"	50.000	ml
Distilled water	950.000	ml

Dissolve the nitritotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8 with NaOH or H₂SO₄.

"Metals 44":

Na-EDTA	250.000	mg
ZnSO ₄ x 7 H ₂ O	1095.000	mg
FeSO ₄ x 7 H ₂ O	500.000	mg
MnSO ₄ x H ₂ O	154.000	mg
CuSO ₄ x 5 H ₂ O	39.200	mg
Co(NO ₃) ₂ x 6 H ₂ O	24.800	mg
Na ₂ B ₄ O ₇ x 10 H ₂ O	17.700	mg
Distilled water	1000.000	ml

Dissolve the EDTA and add a few drops of concentrated H₂SO₄ to retard precipitation of the heavy metal ions.