



ES Medium

Provasoli (1968)

ES Medium is an abbreviated name for enriched natural seawater medium. In addition to nitrate and phosphate, it includes TRIS base buffer, trace metals and vitamins in place of soil water extract. The origin and composition of ES medium is confusing. Recipes for the medium appeared in two 1968 publications (D'Angostino and Provasoli 1968; Provasoli 1968) and the recipes are different. Provasoli himself cited the Provasoli (1968) paper (e.g., D'Agostino and Provasoli 1970; Provasoli et al. 1970; Provasoli and Pintner 1980). Unfortunately, the Provasoli (1968) paper contains several errors. Further confusion arose when some authors (e.g., Starr and Zeikus 1993) attributed ES Medium to Provasoli (1963). but they are incorrect. Provasoli (1963) describes a new enriched natural seawater medium (SWII Medium), derived from Iwasaki's SWI Medium (Iwasaki 1961); however, SWII Medium is not ES Medium. Finally, the PII trace metals solution in ES Medium is not the original formulation (Provasoli 1958). The molar concentrations differ and he substitutes sulfated Mn, Z and Co for the original chlorinated forms. PII trace metals is sometimes traced back to Provasoliet al. (1957) (e.g., by Provasoli himself in D'Angostino and Provasoli 1968), but it first appeared in Provasoli (1958) and subsequently in Provasoli (1963). See Provasoli (1968) for this version of PII trace metals.

In addition to this recipe, and the one by D'Angostino and Provasoli (1968), McLachlin (1973) provides an ES Medium recipe that is different. McLachlin's version (via John West) has 6.6×10^{-5} M TRIS, 6.6×10^{-5} M nitrate, 2.5×10^{-6} M glycerophosphate, 7.2×10^{-3} M iron-EDTA and a different formulation of vitamins and the PII trace metals; i.e., everything is different. West and McBride (1999) provide another version of ES Medium (see below) that is probably the best formulation for general use.

First, prepare and then autoclave the necessary stock solutions, filter sterilize and store refrigerated or frozen (vitamins). To prepare the enrichment stock solution, begin with 900 mL of dH_2O , add the following components (vitamins should be added last after mixing other ingredients), bring the final volume to 1 liter with dH_2O and pasteurize.

To prepare ES Medium, add 20 mL of the enrichment stock solution to 980 mL of filtered natural seawater. Pasteurize.



Enrichment Stock Solution

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
TRIS base		5.0 g	8.26 x 10 ⁻⁴ M
NaNO ₃		3.5 g	8.24 x 10 ⁻⁴ M
Na ₂ b-glycerophosphate H ₂ O		0.5 g	4.63 x 10 ⁻⁵ M
Iron-EDTA Solution	(see recipe below)	250 mL	
PII trace metal solution	(see below)	25 mL	
Thiamine (vit. B_1)		0.500 mg	2.96 x 10 ⁻⁸ M
Biotin (vit. H)	5.0 mg L ⁻¹ dH ₂ O	1 mL	4.09 x 10 ⁻¹⁰ M
Cyanocobalomin (vit. B ₁₂)	10.0 mg L ⁻¹ dH ₂ O	1 mL	1.48 x 10 ⁻¹⁰ M

Iron-EDTA Solution

The original recipe used anhydrous Na_2 EDTA, but because this is difficult to dissolve, we have used 1.274 times as much Na_2 EDTA $2H_2O$ to achieve the same molarity. Begin with 900 mL of dH_2O , dissolve the EDTA and then the iron sulfate. Bring the final volume to 1 liter, sterilize and store refrigerated.

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA • 2H ₂ O		0.841 g	1.13 x 10-5
Fe(NH ₄) ₂ (SO ₄) ₂ • 6H ₂ O		0.702	8.95 x 10-6

PII Trace Metals

Provasoli 1968

The appropriate amount of $Na_2EDTA\ 2H_2O$ is substituted for the original anhydrous $Na_2\ EDTA$. Beginning with 900 mL of dH_2O , dissolve the EDTA and then individually dissolve the following components. (The boron is not necessary for enriching natural seawater and should be left out.) Bring the final volume to 1 liter and store refrigerated.



Component	Stock Solution	Quantity	Molar Concentration in Final Medium
Na ₂ EDTA • 2H ₂ O		12.74 g	1.71 x 10 ⁻⁴ M
FeCl ₃ • 6H ₂ O		0.484 g	8.95 x 10 ⁻⁶ M
H_3BO_3		11.439 g	9.25 x 10 ⁻⁵ M
$MnSO_4 \bullet 4H_2O$		1.624 g	3.64 x 10 ⁻⁵ M
$ZnSO_4 \bullet 7H_2O$		0.220 g	3.82 x 10 ⁻⁶ M
CoSO ₄ • 7H ₂ O		0.048 g	8.48 x 10 ⁻⁷ M

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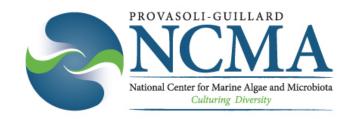
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