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 Provasoli*et 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 x 10^{-5} M TRIS, 6.6 x 10^{-5} M nitrate, 2.5 x 10^{-6} M glycerophosphate, 7.2 x 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.

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Enrichment Stock Solution

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
TRIS base	---	5.0 g	8.26×10^{-4} M
$NaNO_3$	---	3.5 g	8.24×10^{-4} M
Na_2 b-glycerophosphate H_2O	---	0.5 g	4.63×10^{-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	2.96×10^{-8} M
Biotin (vit. H)	5.0 mg L^{-1} dH_2O	1 mL	4.09×10^{-10} M
Cyanocobalomin (vit. B_{12})	10.0 mg L^{-1} dH_2O	1 mL	1.48×10^{-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 $Na_2EDTA\ 2H_2O$ 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
$Na_2EDTA \bullet 2H_2O$	---	0.841 g	1.13 x 10-5
$Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$	---	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
$Na_2EDTA \bullet 2H_2O$	---	12.74 g	1.71×10^{-4} M
$FeCl_3 \bullet 6H_2O$	---	0.484 g	8.95×10^{-6} M
H_3BO_3	---	11.439 g	9.25×10^{-5} M
$MnSO_4 \bullet 4H_2O$	---	1.624 g	3.64×10^{-5} M
$ZnSO_4 \bullet 7H_2O$	---	0.220 g	3.82×10^{-6} M
$CoSO_4 \bullet 7H_2O$	---	0.048 g	8.48×10^{-7} M

References

D'Agostino, A.S. and Provasoli, L. 1968. Effects of salinity and nutrients on mono- and diaxenic cultures of two strains of *Artemia salina*. *Biol. Bull.* 134: 1-14.

D'Agostino, A. and Provasoli, L. 1970. Dixenic culture of *Daphnia magna*, Straus. *Biol. Bull.* **139**: 485-94.

Iwasaki, H. 1961. The life-cycle of *Porphyra tenera* in vitro. *Biol. Bull.* **121**: 173-87.

McLachlan, J. 1973. Growth media - marine. pp. 25-51. *In* Stein, J.R (ed.) *Culture Methods & Growth Measurements*. Cambridge Univ. Press, Cambridge.

Provasoli, L. 1958. Effect of plant hormones on *Ulva*. *Biol. Bull.* **114**: 375-84.

Provasoli, L. 1963. Growing marine seaweeds. pp. 9-17. *In* De Virville, A.D. and Feldmann, J. (eds.) *Proc. 4th Internatl. Seaweed Symp.* Pergamon Press, Oxford.

Provasoli, L. 1968. Media and prospects for the cultivation of marine algae. pp. 63-75. *In* Watanabe, A. and Hattori, A. (Eds.) *Cultures and Collections of Algae.* Proc. U.S.-Japan Conf. Hakone, Japan, September 1966. Publ. by the Jap. Soc. Plant Physiol.

Provasoli, L., Conklin, D.E. and D'Angostino, A.S. 1970. Factors inducing fertility in aseptic Crustacea. *HelgolÃf¤nder wiss. Meeresunters.* **20**: 443-454.

Provasoli, L. and Pintner, I.J. 1980. Bacteria induced polymorphism in an axenic laboratory strain of *Ulva lactuca* (Chlorophyceae). *J. Phycol.* **16**: 196-201.

Starr, R.C. and Zeikus, J.A. 1993. UTEX- the culture collection of algae at the University of Texas at Austin. *J. Phycol.* **29** (suppl.): 1-106.

West, J. A. & McBride, D. L.. 1999. Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). *Hydrobiologia* 298/299: 101-113.