

10 Antibiotic (10AB) stock solution.

This recipe makes up a 10X concentrated solution. The solution should be prepared in 100 mL 0.22 µm filtered seawater (FSW) and diluted 1:10 for use.

Penicillin-G	1.0 g
Streptomycin	2.0 g
Kanamycin	1.0 g
Neomycin	0.2 g
Nystatin	0.015 g
Erythromycin	0.006 g
Gentamycin	0.008 g
Polymixin-B	0.016 g
Tetracycline	0.012 g
Vancomycin	0.012 g

This recipe was published by Polne-Fuller, M. (1991) A novel technique for preparation of axenic cultures of *Symbiodinium* (Pyrrophyta) through selective digestion by amoebae. *Journal of Phycology* 27: 552-554.

f/2 medium* (Guillard and Ryther 1963)

Stock solutions

For each, to 1.0 L of distilled water add:

NaNO ₃	75.0 g
NaH ₂ PO ₄	5.0 g

Trace Metals stock solution

To 1.0 L of distilled water add the following:

Na ₂ EDTA	4.36 g
FeCl ₃ •6H ₂ O	3.15 g
Primary Metals Stocks	1.0 mL of each (recipe below)

Primary Trace Metals stock solutions

To 100 mL of distilled water add the following:

CuSO ₄ •5H ₂ O	1.0 g
ZnSO ₄ •7H ₂ O	2.2 g
CoCl ₂ •6H ₂ O	1.0 g
MnCl ₂ •4H ₂ O	18.0 g
NaMoO ₄ •2H ₂ O	0.63 g

Vitamin Stock solution

Biotin	10.0 mL of 0.1 mg•mL ⁻¹ solution
Vitamin B ₁₂	1.0 mL of 1.0 mg•mL ⁻¹ solution
Thiamine HCl	2.0 g

Final Medium

To 950 mL of 0.22 μM filtered seawater (FSW) add:

NaNO ₃ Stock solution	1.0 mL
NaH ₂ PO ₄ Stock Solution	1.0 mL
Trace Metals Stock Solution	1.0 mL
Vitamin Stock Solution	0.5 mL

Filter sterilize at 0.22 μM before use and store at 4° C.

* Si has been removed from this recipe to reduce the growth of contaminating diatoms.

A historical list of growth media that has been employed in zooxanthellae culturing. We use f/2 medium (Guillard and Ryther 1963) (recipe below)

<u>Name of Medium</u>	<u>Formulation by:</u>	<u>Employed by:</u>
ASP-8A	Ahles 1967	Schoenberg and Trench 1980a Fitt et al. 1981 Colley and Trench 1983 Chang et al. 1983 Fitt and Trench 1983 Trench and Fisher 1983 Fitt 1984 Blank 1987 Trench and Blank 1987 Blank and Trench 1988 Blank and Huss 1989 Rogerson et al. 1989 Lesser and Shick 1990 Markell et al. 1992 Iglesias-Prieto et al. 1992 Markell and Trench 1993 Iglesias-Prieto et al. 1993 Iglesias-Prieto and Trench 1994 Trench and Thinh 1995 Iglesias-Prieto and Trench 1997 Stochaj and Grossman 1997 Wilcox 1998 Warner et al. 1999 Banaszak et al. 2000
EMS	Watanabe et al. 1988	Carlos et al. 1999
K	Keller et al 1987	Carlos et al. 1999 Carlos et al. 2000
M series	McLaughlin and Zahl 1959	McLaughlin and Zahl 1959 Kinzie 1974
CSI	McLaughlin and Zahl 1959	McLaughlin and Zahl 1959

f/2	Guillard and Ryther 1962	Kinzie et al. 1984 Crafts and Tuliszewshi 1995 Rowan et al. 1996 Baille et al. 1998 Belda-Baillie et al. 1999 Santos et al. submitted
ES	Provasoli 1966	Kinzie and Chee 1979 Lerch and Cook 1984 Steen 1987
ISM	Lee et al. 1980	Lee et al. 1995

Please email additional citations, recipes and/or questions to Scott R. Santos at santos@auburn.edu