

Supplementary File 2: Walne's Medium Recipe (Walne, 1970)**a. Composition of Walne's medium in 1000 ml**

Quantity	Component	Stock Solution
1 ml	Nutrient stock solution	(see recipe below)
0.1 ml	Vitamin stock solution	(see recipe below)
Bring up to 1000 ml	Filtered seawater	-

b. Composition of nutrient stock solution in 1000 ml

Component	Quantity used	Concentration in final medium (M)
NaNO ₃	100.00 g	1.18×10^{-3}
H ₃ BO ₃	33.60 g	5.43×10^{-4}
EDTA	45.00 g	1.54×10^{-4}
NaH ₂ PO ₄ ·2H ₂ O	20.00 g	1.28×10^{-4}
FeCl ₃ ·6H ₂ O	1.30 g	4.81×10^{-6}
MnCl ₂ ·4H ₂ O	0.36 g	1.82×10^{-6}
Trace metals solution	1 ml	(see recipe below)
Distilled water	Bring up to 1000 ml	-

c. Composition of trace metals solution in 100 ml

Component	Quantity used	Concentration in final medium (M)
ZnCl ₂	21.00 g	1.54×10^{-7}
CoCl ₂ ·6H ₂ O	20.00 g	8.41×10^{-8}
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	9.00 g	7.28×10^{-9}
CuSO ₄ ·5H ₂ O	20.00 g	8.01×10^{-8}
Distilled water	Bring up to 100 ml	-

d. Composition of vitamin stock solution in 100 ml

Component	Quantity used	Concentration in final medium (M)
Thiamine.HCl (Vitamin B ₁)	1.00 g	2.96×10^{-10}
Cyanocobalamin (B ₁₂)	50.00 mg	3.69×10^{-8}
Distilled water	Bring up to 100 ml	-