

L1++

(Modified from: Guillard R. R. L. and Hargraves P. E., 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycol.* 32: 234-236.)

Filter 1L of natural sea water with GF/F (0.45 μ m) and autoclave for 15 min at 120°C.

After cooling, add the following filter sterilized (0.2 μ m) components to complete the medium:

Minerals	Stock Solutions (g/L)	Quantity (mL Stock/L Media)	Molarity (mM)
NaNO ₃	150	0.5	0.8
NaH ₂ PO ₄ · 2H ₂ O	6.9	0.725	0.03
Na ₂ SiO ₃ · 9H ₂ O	21.3	1.4	0.1
Trace Element Solution	See recipe below	1	-
Vitamins3 Mix	See recipe below	1	-
NH ₄ Cl	26.7	0.3	0.15

For solid medium use 7g/L of agarose. Sterilize the agarose separately in 550 ml of milliQ water. In this case the mineral solution is filled up to 400 ml.

Trace Element Solution:

Trace metals	Stock Solution (g/L mQ)	Quantity used/L
Na ₂ -EDTA · 2H ₂ O	-	4.36 g
FeCl ₃ · 6H ₂ O	-	3.15 g
MnCl ₂ · 4H ₂ O	178.1	1 mL
ZnSO ₄ · 7H ₂ O	2.3	10 mL
CoCl ₂ · 6H ₂ O	1.19	10 mL
CuSO ₄ · 5H ₂ O	2.5	1 mL
Na ₂ MoO ₄ · 2H ₂ O	19.9	1 mL
Na ₂ SeO ₃ · 5H ₂ O	2.63	1 mL
NiSO ₄ · 6H ₂ O	2.63	1 mL
V ₂ O ₅	0.09	10 mL
K ₂ CrO ₄	1.94	1 mL

Vitamins 3 Mix:

Vitamins	Vitamins 3 mix (quantity/100mL)
Thiamine-HCl	10 mg
d-biotin*	100 μ L (from a stock of 5 mg in 10mL)
Vitamin B12	100 μ L (from a stock of 5 mg in 10mL)

*Dissolve first in 0.1 mL 2M NaOH. Then add 9.9 mL of mQ water.