



K/2 Ian / K-ET +2

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ABSTRACT

Modified from K medium by Ian Probert

Reference

Keller, M.D., Selvin, R.C., Claus, W. & Guillard, R.R.L. 1987. Media for the culture of oceanic ultraphytoplankton. J. Phycol. 23:633–8.

GUIDELINES



BEFORE STARTING

Please refer to our general recommendations to grow cultures :

<https://www.protocols.io/private/A48906DC1374AD6281495CB86A8F092F>

- 1 ■ To 994 ml of seawater (pH 8.2, adjusted with NaOH) add:

Quantity	Compound	Stock solution (sterile)	Final conc. in K medium
0.5ml	NaNO ₃	48.9542g/litre H ₂ O	288µM
0.5ml	NH ₄ Cl *	0.535g/litre H ₂ O	5µM
0.5ml	KH ₂ PO ₄	4.8992g/litre H ₂ O	18µM
0.5ml	FeEDTA solution	(see recipe below)	(see below)
0.5ml	Trace metal solution	(see recipe below)	(see below)
1.0ml	f/2 vitamin solution	(see recipe below)	(see below)

* optional

FeEDTA solution

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- To 950ml distilled H₂O add:

Quantity	Compound	Stock solution	Final conc. in K medium
4.3g	(Na)FeEDTA	-	5.85µM

- Make up to 1 litre with milliQ H₂O, sterilize (filter 0.22µm) and store in fridge.

Trace metal solution

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- To 950ml distilled H₂O add:

Quantity	Compound	Stock solution	Final conc. in K medium
37.22g	Na ₂ EDTA.2H ₂ O	-	50µM
1.0ml	CuSO ₄ .5H ₂ O	2.497g/litre H ₂ O	0.005µM
1.0ml	Na ₂ MoO ₄ .2H ₂ O	7.2585g/litre H ₂ O	0.015µM
1.0ml	ZnSO ₄ .7H ₂ O	23.0g/litre H ₂ O	0.004µM
1.0ml	CoSO ₄ .7H ₂ O	14.055g/litre H ₂ O	0.025µM
1.0ml	MnCl ₂ .4H ₂ O	178.11g/litre H ₂ O	0.45µM
1.0ml	H ₂ SeO ₃	1.29g/litre H ₂ O	0.005µM
1.0ml	NiCl ₂ .6H ₂ O	1.49 g/litre H ₂ O	0.00314µM

- Make up to 1 litre with milliQ H₂O, sterilize (filter 0.22µm) and store in fridge.

f/2 Vitamin solution

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- To 950ml distilled H₂O add

Quantity	Compound	Stock solution	Final conc. in K medium
1.0ml	Vit. B ₁₂ (cyanocobalamin)	0.5g/litre H ₂ O	0.37nM
1.0ml	Biotin	5.0mg/litre H ₂ O	2.0nM
100.0mg	Thiamine HCl	-	0.3µM

- Make up to 1 litre with milliQ H₂O, filter sterilize into plastic vials and store in freezer.

Sterilization of medium

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- Optional: Heat to 80°C for 2 hours and leave to cool – this should kill most organisms but should not chemically modify the medium too much
- Filter sterilize through 0.22µm filters (e.g. Millipore Steritop units) into sterile (autoclaved) polycarbonate bottles.

For K-ET

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- Add 10-30 ml marine soil extract (ET)