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K/2 Ian / K-ET V.1

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Protocol status: Working
We use this protocol and it's working

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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.

Before start

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_2O	288µM
	0.5ml	NH_4Cl *	0.535g/litre H_2O	5µM
	0.5ml	KH_2PO_4	4.8992g/litre H_2O	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

2 ▪ To 950ml distilled H_2O add:

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

▪ Make up to 1 litre with milliQ H_2O, sterilize (filter 0.22µm) and store in fridge.

Trace metal solution

3 ▪ To 950ml distilled H_2O add:

	Quantity	Compound	Stock solution	Final conc. in K medium
	37.22 g	$Na_2EDTA.2H_2O$	-	50µM
	1.0ml	$CuSO_4.5H_2O$	2.497 g/litre H_2O	0.005 µM
	1.0ml	$Na_2MoO_4.2H_2O$	7.2585g/litre H_2O	0.015 µM
	1.0ml	$ZnSO_4.7H_2O$	23.0g /litre H_2O	0.004 µM
	1.0ml	$CoSO4.7H_2O$	14.055g/litre H_2O	0.025 µM
	1.0ml	$MnCl_2.4H_2O$	178.11 g/litre H_2O	0.45µM
	1.0ml	H_2SeO_3	1.29g /litre H_2O	0.005 µM
	1.0ml	NiCl2 .6H2 0	1.49 g/litre H_2O	0.00314µM

▪ Make up to 1 litre with milliQ H_2O, sterilize (filter 0.22µm) and store in fridge.

f/2 Vitamin solution

4 ▪ To 950ml distilled H_2O add

	Quantity	Compound	Stock solution	Final conc. in K medium
	1.0ml	Vit. B_{12} (cyanocob	0.5g/litre H_2O	0.37nM

		alamin)		
	1.0ml	Biotin	5.0mg/litre H_2O	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

5
- 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

6
- Add 10-30 ml marine soil extract (ET)