VERSION 2

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 We use this protocol and it's working

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K/2 Ian / K-ET V.2

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Roscoff Culture Collection

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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 INSTRUCTIONS

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

2

- 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

3

- To 950ml distilled H₂O add:

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

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

f/2 Vitamin solution

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- To 950 ml distilled H₂O add

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

- 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) as detailed in the protocol below

Protocol

NAME

Soil extract for algal media

CREATED BY

Daniel Vaultot

PREVIEW

- 6.1 Sample 10 g of dry soil from a place that does not contain any pesticide nor pollutant and where you can be sure to be able to come back in order to always use the same soil. The soil must be dry because the boiling step will be longer if the soil is not dry.
- 6.2 Add the 10 g soil to 400ml of milliQ water.
- 6.3 Boil during 1 hour.
- 6.4 Filter on 0.2 µm or first through 0.8/0.4 µm and then through 0.2µm in order not to clog the filter.

6.5 Aliquot and freeze.

6.6 Add 30 à 50ml of soil extract per 1 L of medium.