Sep 13, 2019 Version 2

K/2 Ian / K-ET V.2

DOI
https://dx.doi.org/10.17504/protocols.io.7bxhipn

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Roscoff Culture Collection

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Protocol Citation: Ian Probert 2019. K/2 Ian / K-ET. **protocols.io** https://dx.doi.org/10.17504/protocols.io.7bxhipn

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

Created: September 13, 2019

Last Modified: September 13, 2019

Protocol Integer ID: 27735

Keywords: oceanic ultraphytoplankton, media for the culture, medium

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	$NaNO_3$	48.9542 g/litre H_2O	288 µM
	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	-	5.85 µM

- 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**
	37.22g	$Na_2EDTA.2H_2O$	-	50µM
	1.0ml	$CuSO_4.5H_2O$	2.497g/litre H_2O	0.005µM
	1.0ml	$Na_2MoO_4.2H_2O$	7.2585g/litre H_2O	0.015µM
	1.0ml	$ZnSO_4.7H_2O$	23.0g/litre H_2O	0.004µM
	1.0ml	CoSO4.7 H_2O	14.055g/litre H_2O	0.025µM
	1.0ml	$MnCl_2.4H_2O$	178.11g/litre H_2O	0.45 µM
	1.0ml	H_2SeO_3	1.29g/litre H_2O	0.005µM

	1.0ml	NiCl 2.6H 20	1.49 g/litre H_2O	0.00 314µM

- 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} (cyanocobalamin)	0.5g /litre H_2O	0.37 nM
	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) as detailed in the protocol below

Protocol

NAME

Soil extract for algal media

CREATED BY

Daniel Vaulot

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.