## K/2(-Tris-Si) (Keller et al., 1987) – modified by I. Probert

To 994 ml of seawater (optional: Heat seawater to 80°C for 2 hours and leave to cool – this should kill most organisms but should not chemically modify the medium too much) add:

Quantity	Compound	Stock solution (sterile)	Final conc. in K medium
O.5ml	NaNO <sub>3</sub>	48.9542g/litre H <sub>2</sub> O	288μM
0.5ml	NH <sub>4</sub> Cl *	0.535g/litre H <sub>2</sub> O	5μΜ
0.5ml	KH <sub>2</sub> PO4	4.8992g/litre H <sub>2</sub> 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)

<sup>\*</sup> optional

#### **FeEDTA solution**

## To 950ml distilled H<sub>2</sub>O add:

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

Make up to 1 litre with milliQ H<sub>2</sub>O, sterilize (filter 0.22µm) and store in fridge.

## **Trace metal solution**

# To 950ml distilled H<sub>2</sub>O add:

Quantity	Compound	Stock solution	Final conc. in K medium
37.22g	Na <sub>2</sub> EDTA.2H <sub>2</sub> O	-	50μM
1.0ml	Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	7.2585g/litre H <sub>2</sub> O	0.015µM
1.0ml	ZnSO <sub>4</sub> .7H <sub>2</sub> O	23.0g/litre H <sub>2</sub> O	0.004µM
1.0ml	CoSO4.7H <sub>2</sub> O	14.055g/litre H <sub>2</sub> O	0.025µM
1.0ml	MnCl <sub>2</sub> .4H <sub>2</sub> O	178.11g/litre H <sub>2</sub> O	0.45μM
1.0ml	H <sub>2</sub> SeO <sub>3</sub>	1.29g/litre H <sub>2</sub> O	0.005µM
1.0ml	NiCl2.6H20	1.49 g/litre H <sub>2</sub> O	0.00314µM

Make up to 1 litre with milliQ  $H_2O$ , sterilize (filter  $0.22\mu m$ ) and store in fridge.

#### f/2 Vitamin solution

To 950ml distilled H<sub>2</sub>O add:

Quantity	Compound	Stock solution	Final conc. in K medium
1.0ml	Vit. B <sub>12</sub> (cyanocobalamin)	0.5g/litre H <sub>2</sub> O	0.37nM
1.0ml	Biotin	5.0mg/litre H <sub>2</sub> 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.

After addition of supplements, adjust pH of medium to 8.2 (with 0.2M solution of NaOH) For K-ET, add 10-30 ml marine soil extract (ET)

Sterilization of medium: Filter sterilize through  $0.22\mu m$  filters (e.g. Millipore Steritop units) into sterile (autoclaved) polycarbonate bottles.