

## fwDiatom Medium (freshwater medium for diatoms)

### incl. 15 psu-swDiatom Medium (brackish seawater medium for diatoms)

For 1000 mL final culture medium as freshwater medium (= fwDiatom medium) add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to 850 mL dd-H<sub>2</sub>O. Add **one component after the other until each one has completely mixed** and finally fill up to 1000 mL.

For final culture medium as 15 psu-seawater medium (= brackish seawater medium) prepare a double strength fwDiatom medium as described above and mix 1:1 (v/v) with 30 psu artificial or natural seawater. Artificial seawater salts mixes are e.g. available from pet shops or dedicated retailers. Either mix sterilised fwDiatom Medium with sterile-filtered seawater (do not autoclave seawater), or sterile-filter after both have been mixed. For marine species, a full seawater diatom medium, e.g. f/2-medium should be used.

All stock solutions can be stored unsterilised at 4 °C. Store vitamin mix (SL 10) at -20 °C.

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	20 mL	Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	0.2 g · 100 mL <sup>-1</sup>	1.70 · 10 <sup>-4</sup> M
SL 2	10 mL	K <sub>2</sub> HPO <sub>4</sub>	0.1 g · 100 mL <sup>-1</sup>	5.74 · 10 <sup>-5</sup> M
SL 3	25 mL	MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.1 g · 100 mL <sup>-1</sup>	1.02 · 10 <sup>-4</sup> M
SL 4	20 mL	Na <sub>2</sub> CO <sub>3</sub>	0.1 g · 100 mL <sup>-1</sup>	1.89 · 10 <sup>-4</sup> M
SL 5	50 mL	Na <sub>2</sub> SiO <sub>3</sub> · 5H <sub>2</sub> O	0.1 g · 100 mL <sup>-1</sup>	2.36 · 10 <sup>-4</sup> M
SL 6	10 mL	ferric-citrate	0.1 g · 100 mL <sup>-1</sup>	4.08 · 10 <sup>-5</sup> M
SL 7	10 mL	citric acid	0.1 g · 100 mL <sup>-1</sup>	5.20 · 10 <sup>-5</sup> M
SL 8	5 mL	micronutrient solution (see below)	see below	
SL 9	30 mL	soil extract (see below)	see below	
SL 10	1 mL	vitamin mix (optional, see below)		

Adjust medium to final pH of 6.5 or as desired with HCl and autoclave at 121 °C for 20 min.

#### SL 8 micronutrient solution

Solution	Volume	Component	Concentration in SL	Conc. in final Medium
Solution I	1 mL	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	0.1 g · 100 mL <sup>-1</sup>	1.74 · 10 <sup>-8</sup> M
	2 mL	MnSO <sub>4</sub> · 4H <sub>2</sub> O <sup>1</sup> (!)	0.1 g · 100 mL <sup>-1</sup>	4.48 · 10 <sup>-8</sup> M
	5 mL	H <sub>3</sub> BO <sub>3</sub>	0.2 g · 100 mL <sup>-1</sup>	8.09 · 10 <sup>-7</sup> M
	5 mL	Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	0.02 g · 100 mL <sup>-1</sup>	1.72 · 10 <sup>-8</sup> M
	5 mL	Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	0.02 g · 100 mL <sup>-1</sup>	2.07 · 10 <sup>-8</sup> M
	1 mL	CuSO <sub>4</sub> · 5H <sub>2</sub> O	0.0005 g · 100 mL <sup>-1</sup>	1.00 · 10 <sup>-10</sup> M
	0.4 g	Na <sub>2</sub> EDTA (Titriplex III)		-
	881 mL	distilled water		
Solution II	0.7 g	FeSO <sub>4</sub> · 7H <sub>2</sub> O		1.26 · 10 <sup>-5</sup> M
	0.4 g	Na <sub>2</sub> EDTA (Titriplex III)		-
	100 mL	distilled water		

Prepare and autoclave solutions I and II separately, let both cool down and then pool.

#### SL 9 soil extract

For optimal maintenance of algal stock cultures this medium is modified by the addition of soil extract (SL 9). The soil extract often helps to culture species which are otherwise often hard to culture, but can be left out for mass culturing.

SL 9 (soil extract) 30 mL garden or deciduous forest soil (no fertilisers or pesticides)

Boil 50 g soil in 500 mL distilled water for 5 minutes, let sediment, decant supernatant and centrifuge (15 min. at 5500 rpm), then filter through 1.2-3 µm filter until clear. Tyndallize (important to kill fungal spores!): heat the extract to 100 °C for 15-30 min., then rapidly cool to room temperature and let stand for 24 h; repeat this two more times on consecutive days. Finish by one autolave cycle (121 °C for 30 min.). Store at +4 °C.

#### SL 10 vitamin mix

The addition a vitamin mix is advised for long time life cultures as some algal species need one or two of the vitamins contained in the mix, however, it can be left out for mass culturing. Combine the below amounts together in 100 mL of distilled water. 1 mL aliquots can be stored aseptically and added to the medium aseptically if required.

SL 10	1 mL	Vit. B <sub>1</sub> (Thiamine HCl)	0.1 g · 100 mL <sup>-1</sup>	2.97 · 10 <sup>-6</sup> M
Vitamin mix		Vit. H (Biotin)	0.025 mg · 100 mL <sup>-1</sup>	1.02 · 10 <sup>-9</sup> M
		Vit. B <sub>12</sub> (Cyanocobalamin)	0.015 mg · 100 mL <sup>-1</sup>	1.11 · 10 <sup>-10</sup> M

For storage acidify to a pH of 4.5-5.0 and autoclave, or dispense aseptically through 0.2 µm sterile filters in plastic containers (reaction vials, cryovials, polycarbonate tubes) in 1 mL aliquots and **add aseptically to prepared medium after autoclaving and cooling**. Store at -20 °C.

For stock cultures on agar slants add 1.0-1.3 % Agar (e.g. purified high strength, 1000 g · cm<sup>-2</sup>) to prepared medium before autoclaving.

<sup>1</sup> at CCCryo 0.1 g MnSO<sub>4</sub> · 4H<sub>2</sub>O is replaced by 0.0755 g MnSO<sub>4</sub> · H<sub>2</sub>O