

## MiEg Medium (*Euglena* medium with minerals)

For 1000 mL final culture 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.

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

*Euglena* medium contains per litre:

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 1	10 mL	Na-acetate	10 g · 100 mL <sup>-1</sup>	1.22 · 10 <sup>-2</sup> M
SL 2	10 mL	beef extract	10 g · 100 mL <sup>-1</sup>	n.a.
SL 3	10 mL	bacto-tryptone	10 g · 100 mL <sup>-1</sup>	n.a.
SL 4	10 mL	yeast extract	10 g · 100 mL <sup>-1</sup>	n.a.
SL 5	30 mL	soil extract	n.a.	n.a.

For optimal maintenance of algal stock cultures this BBM is modified by the addition of soil extract (SL 5). The soil extract often helps to culture species which are otherwise often hard to culture, but can be left out for mass culturing.

For **SL 5** (soil extract) 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.

**MiEg-Medium** contains in addition mineral solutions as follows (add to above preparation of 1 litre):

Stock Solution (SL)	Volume	Component	Concentration in SL	Conc. in final Medium
SL 6	20 mL	KNO <sub>3</sub>	1 g · 100 mL <sup>-1</sup>	1.96 · 10 <sup>-4</sup> M
SL 7	10 mL	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	0.2 g · 100 mL <sup>-1</sup>	1.50 · 10 <sup>-5</sup> M
SL 8	10 mL	MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.1 g · 100 mL <sup>-1</sup>	4.00 · 10 <sup>-5</sup> M
SL 9	20 mL	CaSO <sub>4</sub>	saturated solution	

**Adjust to final pH as indicated** in database and autoclave (20 min at 121 °C).

The addition a vitamin mix is advised as some algal species need one or two of the vitamins contained in the mix.

SL 10		Vit. B <sub>1</sub> (Thiamine HCl)	0.1 g · 100 mL <sup>-1</sup>	2.96 · 10 <sup>-6</sup> M
Vitamin mix	1 mL	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.