# **Bold 3N**

NaNO <sub>3</sub>	75 mg
CaCl <sub>2</sub> · 2H <sub>2</sub> O	2.5 mg
MgSO <sub>4</sub> · 7H <sub>2</sub> O	7.5 mg
K <sub>2</sub> HPO <sub>4</sub>	7.5 mg
KH <sub>2</sub> PO <sub>4</sub> <sup>1)</sup>	17.5 mg
NaCl	2.5 mg
Vitamin B <sub>12</sub> <sup>2)</sup>	0. 015 μg
PIV metals	0.6 mL
Soil extract	4 mL
Distilled water	96.4 mL

- 1) In the NIES-Collection, the amount of KH<sub>2</sub>PO<sub>4</sub> is reduced from 17.5 mg to 10.5 mg.
- 2) In the NIES-Collection, the amount of vitamin  $B_{12}$  is increased from 0.015  $\mu g$  to 0.02  $\mu g$ .

### P IV metals

Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	100 mg
FeCl <sub>3</sub> ⋅6H <sub>2</sub> O	19.6 mg
MnCl <sub>2</sub> · 4H <sub>2</sub> O	3.6 mg
ZnCl <sub>2</sub> <sup>1)</sup>	1.04 mg
CoCl <sub>2</sub> · 6H <sub>2</sub> O	0.4 mg
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	0.25 mg
Distilled water	100 mL

1) In the NIES-Collection, 1.04 mg ZnCl<sub>2</sub> is replaced by 2.2mg ZnSO<sub>4</sub>  $\cdot$  7H<sub>2</sub>O.

## Reference

Provasoli, L., Pintner, I. J. 1959 Artificial media for fresh-water algae: problems and suggestions. In *The Ecology of Algae. Spec. Pub. No. 2.*, Eds. by Tryon, C. A., Jr. & Hartmann, R. T., Pymatuning Laboratory of Field Biology, University of Pittsburgh, Pittsburgh, p. 84-96.

#### Soil extract

To 1000 mL distilled water add 200 mL of soil (soil from undisturbed deciduous woodland is best) and heat by autoclaving for 1 h at 105°C. When cool, heat by autoclaving for 1 h at 105°C again. Pass the supernatant through a GF/C filter and Celite, and then pass the filtrate through a GF/F filter. Adjust to 1000 mL by adding distilled water. Dispense 10 mL of the final filtrate into each test tube and sterilize by autoclaving for 20min at 121°C. Keep in a cool place.

### Reference

Provasoli, L., McLaughlin, J. J. A., Droop, M. R. 1957 The development of artificial media for marine algae. *Arch. Mikrobiol.*, **25**, 392-428.