



# AF-6 (pH 3) Medium

Freshwater medium adapted for *Chlamydomonas acidophila*

Reference: Provasoli, L. and Pinter, I. J. (1959). Artificial media for fresh-water algae: problems and suggestions. In: Tryon, C. A., Jr. and Hartmann, R. T. (eds.) The Ecology of Algae. Spec. Pub. No. 2, Pymatuning Laboratory of Field Biology, Pittsburgh pp 84-96.

| STOCK SOLUTIONS                         | CONCENTRATION:<br>g L <sup>-1</sup> DEIONISED WATER (dH <sub>2</sub> O) | QUANTITY FOR MEDIUM |
|---|---|---------------------|
| 1. MES                                  | <i>add reagent directly to medium</i>                                   | 400 mg              |
| 2. NaNO <sub>3</sub>                    | 140 g   | 1.0 mL              |
| 3. NH <sub>4</sub> NO <sub>3</sub>      | 20 g  | 1.1 mL              |
| 4. MgSO <sub>4</sub> .7H <sub>2</sub> O | 80 g  | 0.375 mL            |
| 5. KH <sub>2</sub> PO <sub>4</sub>      | 10 g  | 1.0 mL              |
| 6. K <sub>2</sub> HPO <sub>4</sub>      | 5 g   | 1.0 mL              |
| 7. CaCl <sub>2</sub> .2H <sub>2</sub> O | 29.4 g  | 0.34 mL             |
| 8. Fe citrate                           | <i>see recipe below</i>   | 0.222 mL            |
| 9. Biotin                               | 0.1 g   | 20 µL               |
| 10. Thiamine HCl                        | 0.1 g   | 0.1 mL              |
| 11. Vitamin B <sub>6</sub>              | 0.1 g   | 10 µL               |
| 12. Vitamin B <sub>12</sub>             | 0.1 g   | 10 µL               |
| 13. P IV Metal Mix                      | <i>see recipe below</i>   | 5.0 mL              |

Store all stock solutions in the refrigerator.

## Fe citrate solution

Add both constituents to 1 L of dH<sub>2</sub>O and autoclave to dissolve. Store solution in the dark.

| CONSTITUENT    | QUANTITY |
|----------------|----------|
| Ferric citrate | 9.0 g    |
| Citric acid    | 9.0 g    |

## P IV Metal Mix

Add EDTA to 1 L of dH<sub>2</sub>O and dissolve, then add other constituents to dissolve and autoclave to sterilise. Store solution in the dark.

| CONSTITUENT   | QUANTITY |
|---|----------|
| Na <sub>2</sub> EDTA.2H <sub>2</sub> O              | 1.0 g    |
| FeCl <sub>3</sub> .6H <sub>2</sub> O                | 196 mg   |
| MnCl <sub>2</sub> .4H <sub>2</sub> O                | 36.0 mg  |
| ZnSO <sub>4</sub> .7H <sub>2</sub> O                | 22.0 mg  |
| CoCl <sub>2</sub> .6H <sub>2</sub> O                | 4.0 mg   |
| Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O | 2.5 mg   |

### To prepare AF-6 (pH 3) Medium (1 L)

- Add MES to 990 mL dH<sub>2</sub>O and dissolve.
- Add each stock solution (**2 – 13**) in the stated volume to the MES solution.
- Adjust the pH to 3 with HCl (diluted to 20%).
- Autoclave at 121°C (15 psi for 20 mins).

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