BBM Medium (Bold’s Basal Medium + soil extract + vitamins)

For optimal maintenance of algal stock cultures this BBM is modified by the addition of soil extract (SL 11) and a vitamin mix (SL 12). The soil extract often helps to culture species which are otherwise often hard to culture, but can be left out for mass culturing.

Boil 50 g soil (e.g. unfertilised garden soil) in 500 mL distilled water for 5 minutes, let sediment, decant supernatant and centrifuge (15 min. at 5500 rpm), and filter through a 3 μm filter until clear. Tyndallize 3x at an interval of 24 h to kill fungal and bacterial spores (heat the extract to 100 °C for 15-30 min., then rapidly cool to room temperature, repeat this on three consecutive days). Finish by one autoclave cycle (121 °C for 30 min.). Store at +4 °C.

Adjust medium to final pH of 5.5 / 6.5 or as desired with HCl and autoclave at 121 °C for 20 min.

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

For 1000 mL final culture medium add the following quantities (Volume) of stock solutions (SL) prepared at the given concentrations to 850 mL distilled water. 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 sterile filtered vitamin mix (SL 12) at -20 °C.

<table>
<thead>
<tr>
<th>Stock Solution (SL)</th>
<th>Volume</th>
<th>Component</th>
<th>Concentration in SL</th>
<th>Conc. in final Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL 1</td>
<td>10 mL</td>
<td>NaNO₃</td>
<td>2.50 g · 100 mL⁻¹</td>
<td>2.94 · 10⁻¹ M</td>
</tr>
<tr>
<td>SL 2</td>
<td>10 mL</td>
<td>MgSO₄ · 7H₂O</td>
<td>0.75 g · 100 mL⁻¹</td>
<td>3.04 · 10⁻¹ M</td>
</tr>
<tr>
<td>SL 3</td>
<td>10 mL</td>
<td>NaCl</td>
<td>0.25 g · 100 mL⁻¹</td>
<td>4.28 · 10⁻¹ M</td>
</tr>
<tr>
<td>SL 4</td>
<td>10 mL</td>
<td>K₂HPO₄</td>
<td>0.75 g · 100 mL⁻¹</td>
<td>4.31 · 10⁻¹ M</td>
</tr>
<tr>
<td>SL 5</td>
<td>10 mL</td>
<td>KH₂PO₄</td>
<td>1.75 g · 100 mL⁻¹</td>
<td>1.29 · 10⁻¹ M</td>
</tr>
<tr>
<td>SL 6</td>
<td>10 mL</td>
<td>CaCl₂ · 2H₂O</td>
<td>0.25 g · 100 mL⁻¹</td>
<td>1.70 · 10⁻⁴ M</td>
</tr>
<tr>
<td>SL 7</td>
<td>1 mL</td>
<td>ZnSO₄ · 7H₂O</td>
<td>8.82 g · L⁻¹</td>
<td>3.07 · 10⁻⁴ M</td>
</tr>
<tr>
<td>Trace elements</td>
<td>1 mL</td>
<td>MnCl₂ · 4H₂O</td>
<td>1.44 g · L⁻¹</td>
<td>7.28 · 10⁻⁴ M</td>
</tr>
<tr>
<td>Solution</td>
<td></td>
<td>MoO₃</td>
<td>0.71 g · L⁻¹</td>
<td>4.93 · 10⁻⁴ M</td>
</tr>
<tr>
<td>SL 8</td>
<td>1 mL</td>
<td>CuSO₄ · 5H₂O</td>
<td>1.57 g · L⁻¹</td>
<td>6.29 · 10⁻⁶ M</td>
</tr>
<tr>
<td>EDTA-KOH Solution</td>
<td>1 mL</td>
<td>Co(NO₃)₂ · 6H₂O</td>
<td>0.49 g · L⁻¹</td>
<td>1.68 · 10⁻⁶ M</td>
</tr>
<tr>
<td>SL 9</td>
<td>1 mL</td>
<td>H₂BO₃</td>
<td>1.14 g · 100 mL⁻¹</td>
<td>1.85 · 10⁻¹ M</td>
</tr>
<tr>
<td>EDTA-KOH Solution</td>
<td>1 mL</td>
<td>Na₂EDTA · 2H₂O (Titriplex III)</td>
<td>5.0 g · 100 mL⁻¹</td>
<td>1.71 · 10⁻¹ M</td>
</tr>
<tr>
<td>SL 10</td>
<td>1 mL</td>
<td>KOH</td>
<td>3.1 g · 100 mL⁻¹</td>
<td>5.53 · 10⁻¹ M</td>
</tr>
<tr>
<td>Ferric Solution</td>
<td>1 mL</td>
<td>FeSO₄ · 7H₂O</td>
<td>4.98 g · L⁻¹</td>
<td>1.79 · 10⁻¹ M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H₂SO₄ conc.</td>
<td>1 mL (to acidify)</td>
<td></td>
</tr>
</tbody>
</table>

Combine all trace elements in one SL. Dissolve each component completely one after the other. It may need autoclaving to dissolve. Trace elements solution should not be stored in glass containers, but instead in teflon or polycarbonate containers to prevent adsorption of metals to container surface.

This results in the original Bold’s Basal Medium (BBM) according to Bischoff & Bold (1963). The pH-value will be about 6.4 to 6.8 at a conductivity of 1.4 mS cm⁻¹.

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⁻²) to prepared medium before autoclaving.


