**Bold 3N Medium Recipe**

**Directions**

Modification of Bold's recipe. General purpose freshwater medium used for xenic cultures, especially blue-greens and reds.

For 1 L Total
1. To approximately 850 mL of dH₂O, add each of the components in the order specified (except vitamins) while stirring continuously.
2. Bring the total volume to 1 L with dH₂O.
   *For 1.5% agar medium add 15 g of agar into the flask; do not mix.
3. Cover and autoclave medium.
4. When cooled add Vitamin B₁₂.
   *For agar medium add vitamin, mix, and dispense before agar solidifies.
5. Store at refrigerator temperature.

<table>
<thead>
<tr>
<th>#</th>
<th>Component</th>
<th>Amount</th>
<th>Stock Solution Concentration</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaNO₃ (Fisher BP360-500)</td>
<td>30 mL/L</td>
<td>10 g/400mL dH₂O</td>
<td>8.82 mM</td>
</tr>
<tr>
<td>2</td>
<td>CaCl₂·2H₂O (Sigma C-3881)</td>
<td>10 mL/L</td>
<td>1 g/400mL dH₂O</td>
<td>0.17 mM</td>
</tr>
<tr>
<td>3</td>
<td>MgSO₄·7H₂O (Sigma 230391)</td>
<td>10 mL/L</td>
<td>3 g/400mL dH₂O</td>
<td>0.3 mM</td>
</tr>
<tr>
<td>4</td>
<td>K₂HPO₄ (Sigma P 3786)</td>
<td>10 mL/L</td>
<td>3 g/400mL dH₂O</td>
<td>0.43 mM</td>
</tr>
<tr>
<td>5</td>
<td>KH₂PO₄ (Sigma P 0662)</td>
<td>10 mL/L</td>
<td>7 g/400mL dH₂O</td>
<td>1.29 mM</td>
</tr>
<tr>
<td>6</td>
<td>NaCl (Fisher S271-500)</td>
<td>10 mL/L</td>
<td>1 g/400mL dH₂O</td>
<td>0.43 mM</td>
</tr>
<tr>
<td>7</td>
<td>P-IV Metal Solution</td>
<td>6 mL/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Soilwater: GR+ Medium</td>
<td>40 mL/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Vitamin B₁₂</td>
<td>1 mL/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>