Artificial Seawater Based AMP1 Medium

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Works for me

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LAST MODIFIED
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PROTOCOL INTEGER ID
1037

GUIDELINES

Turk's Island Salt Mix

<table>
<thead>
<tr>
<th>Chemical</th>
<th>g/2 L</th>
<th>Final Conc.</th>
<th>Mfc.-PN</th>
<th>Chemical Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>56.22</td>
<td>481 mM</td>
<td>Fisher S271</td>
<td>ACS</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>13.8</td>
<td>28 mM</td>
<td>Sigma M2773</td>
<td>Mo.Biol. 99%</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>10.98</td>
<td>27 mM</td>
<td>AlfaAesar</td>
<td>99.99%</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>2.94</td>
<td>10 mM</td>
<td>Fisher C79</td>
<td>ACS</td>
</tr>
<tr>
<td>KCl</td>
<td>1.34</td>
<td>9 mM</td>
<td>Acros</td>
<td>99+%</td>
</tr>
<tr>
<td>MQ·H₂O</td>
<td>na</td>
<td>in house</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

Macronutrients

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### Macronutrient

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>g/100 ml</th>
<th>Final Conc.</th>
<th>Mfc.-PN</th>
<th>Chemical Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M NaH$_2$PO$_4$·H$_2$O</td>
<td>1.38</td>
<td>50 µM</td>
<td>Mallinckrodt 7892-04</td>
<td>ACS</td>
</tr>
<tr>
<td>0.8 M (NH$_4$)$_2$SO$_4$</td>
<td>10.57</td>
<td>400 µM</td>
<td>VWR-BDH 0216</td>
<td>ACS 99%</td>
</tr>
</tbody>
</table>

### Buffers

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Stock</th>
<th>Final Conc.</th>
<th>Mfc.-PN</th>
<th>Chemical Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 M NaHCO$_3$</td>
<td>0.504 g/10 ml</td>
<td>6 mM</td>
<td>Sigma S6014</td>
<td>ACS 99.7-100.3%</td>
</tr>
<tr>
<td>1 M HEPES</td>
<td>11.9 g/50 ml</td>
<td>1 mM</td>
<td>Fisher BP310</td>
<td>Mol. Biol.</td>
</tr>
</tbody>
</table>

### Trace Metal Mix Working Stock

<table>
<thead>
<tr>
<th>Trace Metal</th>
<th>Primary Stock</th>
<th>Final Conc.</th>
<th>Mfc.-PN</th>
<th>Chemical Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$EDTA·2H$_2$O</td>
<td>0.2175 g/50 ml</td>
<td>0.1170 µM</td>
<td>Sigma E4884</td>
<td>ACS 99-101%</td>
</tr>
<tr>
<td>FeCl$_3$·6H$_2$O</td>
<td>0.16 g/50 ml</td>
<td>0.1180 µM</td>
<td>Sigma 44944</td>
<td>ACS 98-102%</td>
</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O</td>
<td>1.15 g/50 ml</td>
<td>0.0008 µM</td>
<td>Sigma 204986</td>
<td>&gt;99.5%</td>
</tr>
<tr>
<td>CoCl$_2$·6H$_2$O</td>
<td>0.595 g/50 ml</td>
<td>0.0005 µM</td>
<td>Sigma 60820</td>
<td>&gt;98%</td>
</tr>
<tr>
<td>MnCl$_2$·4H$_2$O</td>
<td>8.905 g/50 ml</td>
<td>0.0090 µM</td>
<td>M3634</td>
<td>ReagentPlus &gt;99%</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$·2H$_2$O</td>
<td>0.363 g/50 ml</td>
<td>0.0003 µM</td>
<td>M1651</td>
<td>&gt;99.5%</td>
</tr>
<tr>
<td>Na$_2$SeO$_3$</td>
<td>0.865 g/50 ml</td>
<td>0.0010 µM</td>
<td>S5261</td>
<td>BioReagent ~98%</td>
</tr>
<tr>
<td>NiCl$_2$·6H$_2$O</td>
<td>1.19 g/50 ml</td>
<td>0.0010 µM</td>
<td>223387</td>
<td>ReagentPlus</td>
</tr>
</tbody>
</table>

Note: Use primary stocks of ZnSO$_4$, CoCl$_2$, MnCl$_2$, Na$_2$MoO$_4$, Na$_2$SeO$_3$, and NiCl$_2$ prepared for Pro99 trace metal working stock.
**MATERIALS**

- Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma Aldrich Catalog #E4884
- Sodium Chloride Fisher Scientific Catalog #S271
- Magnesium sulfate heptahydrate Sigma Aldrich Catalog #M2773
- Calcium Chloride Dihydrate Fisher Scientific Catalog #C79
- Sodium bicarbonate Sigma Aldrich Catalog #S6014
- HEPES Fisher Scientific Catalog #BP310
- Iron(III) chloride hexahydrate Sigma Aldrich Catalog #44944
- Zinc sulfate heptahydrate Sigma Aldrich Catalog #204986
- Cobalt(II) chloride hexahydrate Sigma Aldrich Catalog #60820
- Manganese(II) chloride tetrahydrate Sigma Aldrich Catalog #M3634
- Sodium molybdate dihydrate Sigma Aldrich Catalog #M1651
- Sodium selenite Sigma Aldrich Catalog #S5261
- Nickel(II) chloride hexahydrate Sigma Aldrich Catalog #223387

**STEP MATERIALS**

- HEPES Fisher Scientific Catalog #BP310
- Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma Aldrich Catalog #E4884
- Iron(III) chloride hexahydrate Sigma Aldrich Catalog #44944
- HEPES Fisher Scientific Catalog #BP310
- Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma Aldrich Catalog #E4884
- Iron(III) chloride hexahydrate Sigma Aldrich Catalog #44944

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Turk's Island Salt Mix

1. Dissolve each salt completely before adding the next one. Refer to table in guidelines for full list of salts.

2. Dispense into 500 ml acid-washed polycarbonate bottles.

3. Autoclave 30 min. 

Macronutrients

4. pH NaH$_2$PO$_4$ to 7.5 using 1M NaOH

   About 8ml for 100ml volume.

   Prepare each one separately.

5. Filter sterilize each solution using 0.2 µm syringe filter into new, sterile 50ml centrifuge tubes or acid washed and sterile polycarbonate bottles.

6. Store at 4°C

   Dispense 250 µl of each per 500 ml bottle of Turk's Island Salt Mix when preparing final medium.

Buffers

7. pH HEPES to 7.5 using 1M NaOH

   About 9 ml for 50 ml volume.

   Prepare each one separately.

   ![HEPES Fisher](https://www.fisherscientific.com/)

   Scientific Catalog #BP310

8. Filter sterilize each solution using 0.2 µm syringe filter into new, sterile 15 or 50 ml centrifuge tubes or acid washed and sterile polycarbonate bottles.

9. Store at 4°C

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The NaHCO$_3$ should be made monthly.

Dispense 5 ml of NaHCO$_3$ and 0.5 ml HEPES per 500 ml bottle of Turk's Island Salt Mix when preparing final medium.

**Trace Metal Mix Working Stock**

11. Weigh out 0.2175 g Na$_2$EDTA·2H$_2$O using dust free paper

   Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma
   Aldrich Catalog #E4884

12. Transfer to acid washed 50 ml volumetric flask filled with 40 ml MQ-water

13. Dissolve EDTA by inverting flask several times

   May have to heat 5 min. at 80°C to dissolve.

14. Weigh out 0.16 g FeCl$_3$·6H$_2$O using dust free paper

   Iron(III) chloride hexahydrate Sigma
   Aldrich Catalog #44944

15. Dissolve iron chloride into same volumetric flask by inverting several times

16. Individually add and dissolve 50 µl each of the ZnSO$_4$, CoCl$_2$, MnCl$_2$, Na$_2$MoO$_4$, NaSeO$_3$, and NiCl$_2$ Primary Trace Metal Stocks

17. Adjust volume to 50 ml mark with MQ-water

18. Filter through a 0.2 µm syringe filter into sterile, acid washed container in laminar flow hood

19. Store sterile stock at 4°C

20. Dispense 5 µl Stock Trace Metal Mix to 500 ml bottle of Turk's Island Salt Mix when preparing final medium.