Artificial Seawater Based AMP1 Medium

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LAST MODIFIED

Mar 17, 2018

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 M2773</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>OS to 2000 ml</td>
<td>na in house</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

Macronutrients
<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 NaH₂PO₄.H₂O</td>
<td>1.38</td>
<td>50 µM</td>
<td>Mallinckrodt 7892-04</td>
<td>ACS</td>
</tr>
<tr>
<td>0.8 M (NH₄)₂SO₄</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 NaHCO₃</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>Na₂EDTA·2H₂O</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>FeCl₂·6H₂O</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>ZnSO₄·7H₂O</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>CoCl₂·6H₂O</td>
<td>0.595 g/50 ml</td>
<td>0.0005 µM</td>
<td>Sigma 60820</td>
<td>&gt;98%</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>8.905 g/50 ml</td>
<td>0.0090 µM</td>
<td>M3634</td>
<td>ReagentPlus &gt;99%</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.363 g/50 ml</td>
<td>0.0003 µM</td>
<td>M1651</td>
<td>&gt;99.5%</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>0.865 g/50 ml</td>
<td>0.0010 µM</td>
<td>S5261</td>
<td>BioReagent ~98%</td>
</tr>
<tr>
<td>NiCl₂·6H₂O</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 ZnSO₄, CoCl₂, MnCl₂, Na₂MoO₄, Na₂SeO₃, and NiCl₂ prepared for Pro99 trace metal working stock.
Citation: Bonnie Poulos (01/04/2016). Artificial Seawater Based AMP1 Medium. https://dx.doi.org/10.17504/protocols.io.c9mz45

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MATERIALS

**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 | Step 7
- Iron(III) chloride hexahydrate Sigma
  - Aldrich Catalog #44944 | Step 14
- 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 | Step 7
- Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma
  - Aldrich Catalog #E4884
- Iron(III) chloride hexahydrate Sigma
  - Aldrich Catalog #44944 | Step 14
- HEPES Fisher
  - Scientific Catalog #BP310 | Step 7
- Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma
  - Aldrich Catalog #E4884
- Iron(III) chloride hexahydrate Sigma
  - Aldrich Catalog #44944 | Step 14
1. Dissolve each salt completely before adding the next one. Refer to the table in guidelines for a full list of salts.

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

3. Autoclave 30 min. 00:30:00

Macronutrients

4. pH NaH₂PO₄ to 7.5 using 1M NaOH

   About 8 ml for 100 ml volume.

   Prepare each one separately.

5. Filter sterilize each solution using a 0.2 µm syringe filter into new, sterile 50 ml 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 the 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
   Scientific Catalog #BP310

8. Filter sterilize each solution using a 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
The NaHCO₃ should be made monthly.

Dispense 5 ml of NaHCO₃ 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 Na₂EDTA · 2H₂O using dust free paper

|化学物||Sigma Aldrich Catalog # E4884|
|---|---|
|0 g|Ethylenediaminetetraacetic acid disodium salt dihydrate|

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 FeCl₃·6H₂O using dust free paper

|化学物||Sigma Aldrich Catalog # 44944|
|---|---|
|0 µl|Iron(III) chloride hexahydrate|

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

16 Individually add and dissolve 50 µl each of the ZnSO₄, CoCl₂, MnCl₂, Na₂MoO₄, NaSeO₃, and NiCl₂ 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

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