Medium HH
(1 liter)

Step I

600 ml deionized H$_2$O
10 mg EDTA
0.5 g KH$_2$PO$_4$
200 mg MgSO$_4$ · 7 H$_2$O
75 mg CaCl$_2$ · 2 H$_2$O
0.5 g NH$_4$Cl
1 g sodium acetate
0.1 g yeast extract
20 μg Vitamin B$_{12}$
1 ml trace elements
1 g Na pyruvate
1.63 g (10mM) bicine (Sigma)

Autoclave liquid mixture (25 minutes) in dispensing jar containing a dispensing bell connected to the jar by sterile rubber tubing.

Step II

2.5 g NaHCO$_3$
2.5 g Na$_2$CO$_3$
300 ml deionized H$_2$O

Autoclave carbonate/bicarbonate as a dry powder in a 500-ml bottle. After cooling and when you ready to assemble the medium, dissolve the powders in the 300 ml of sterile water.

Step III

0.6 g Na$_2$S · 9 H$_2$O
100 ml degassed (boiled) H$_2$O

Wash crystals of Na$_2$S · 9 H$_2$O in distilled water, dry on a towel, and weigh out. Dissolve the washed crystals in boiling d-H$_2$O and autoclave immediately.

Step IV

After cooling, add dissolved carbonate/bicarbonate (Step II) and sulfide (Step III) mixtures to the sterile bell jar medium (Step I), adjust the pH to 9.0 and immediately dispense into 17-ml screw-cap tubes. Make sure the tubes are completely filled with medium (leaving as small an air bubble as possible) and tightly capped. The final medium may form a slight greyish-black precipitate. Let the medium “age” for at least two days before using.
1 Trace elements (per liter of distilled water):

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
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<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>5.2 g</td>
<td>Na₂MoO₄ · 2H₂O</td>
<td>188 mg</td>
</tr>
<tr>
<td>CoCl₂ · 6 H₂O</td>
<td>190 mg</td>
<td>NiCl₂ · 6H₂O</td>
<td>25 mg</td>
</tr>
<tr>
<td>MnCl₂ · 4H₂O</td>
<td>100 mg</td>
<td>ZnCl₂</td>
<td>70 mg</td>
</tr>
<tr>
<td>FeCl₂ · 4H₂O</td>
<td>1.5 g</td>
<td>VoSO₄ · 2H₂O</td>
<td>30 mg</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>6 mg</td>
<td>Na₂WO₄ · 2H₂O</td>
<td>2 mg</td>
</tr>
<tr>
<td>CuCl₂ · 2H₂O</td>
<td>17 mg</td>
<td>NaHSeO₃</td>
<td>2 mg</td>
</tr>
</tbody>
</table>

Note to users: Any trace elements solution will likely work