120d: METHANOSARCINA MARINE MEDIUM

\[
\begin{align*}
K_2HPO_4 & \quad 0.35 \text{ g} \\
KH_2PO_4 & \quad 0.23 \text{ g} \\
NH_4Cl & \quad 0.50 \text{ g} \\
MgCl_2 \times 6 \text{H}_2\text{O} & \quad 10.00 \text{ g} \\
CaCl_2 \times 2 \text{H}_2\text{O} & \quad 0.15 \text{ g} \\
NaCl & \quad 23.00 \text{ g} \\
\text{FeSO}_4 \times 7 \text{H}_2\text{O solution (0.1% w/v)} & \quad 2.00 \text{ ml} \\
\text{Trace element solution SL-10} & \quad 1.00 \text{ ml} \\
\text{Sodium resazurin (0.1% w/v)} & \quad 0.50 \text{ ml} \\
Na_2CO_3 & \quad 1.00 \text{ g} \\
\text{Methanol (50% v/v)} & \quad 20.00 \text{ ml} \\
\text{Wolin's vitamin solution (10x)} & \quad 1.00 \text{ ml} \\
\text{L-Cysteine HCl \times H}_2\text{O} & \quad 0.30 \text{ g} \\
\text{Na}_2\text{S \times 9 H}_2\text{O} & \quad 0.30 \text{ g} \\
\text{Distilled water} & \quad 1000.00 \text{ ml}
\end{align*}
\]

Dissolve ingredients (except carbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N\textsubscript{2} gas atmosphere as concentrated solutions in tightly closed tubes. Carbonate is prepared under 80% N\textsubscript{2} and 20% CO\textsubscript{2} gas mixture and autoclaved separately. Vitamins are prepared under 100% N\textsubscript{2} gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

\[
\text{FeSO}_4 \times 7 \text{H}_2\text{O solution (0.1% w/v) (from medium 119)}
\]

\[
\begin{align*}
\text{FeSO}_4 \times 7 \text{H}_2\text{O} & \quad 1.00 \text{ g} \\
\text{H}_2\text{SO}_4 \text{ (0.1 N)} & \quad 1000.00 \text{ ml}
\end{align*}
\]

The ferrous sulfate solution is not stable and should be freshly prepared.

\[
\text{Trace element solution SL-10 (from medium 320)}
\]

\[
\begin{align*}
\text{HCl (25%)} & \quad 10.00 \text{ ml} \\
\text{FeCl}_2 \times 4 \text{H}_2\text{O} & \quad 1.50 \text{ g} \\
\text{ZnCl}_2 & \quad 70.00 \text{ mg} \\
\text{MnCl}_2 \times 4 \text{H}_2\text{O} & \quad 100.00 \text{ mg} \\
\text{H}_3\text{BO}_3 & \quad 6.00 \text{ mg}
\end{align*}
\]
### Microorganisms

**120d: METHANOSARCINA MARINE MEDIUM**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCl₂ x 6 H₂O</td>
<td>190.00 mg</td>
</tr>
<tr>
<td>CuCl₂ x 2 H₂O</td>
<td>2.00 mg</td>
</tr>
<tr>
<td>NiCl₂ x 6 H₂O</td>
<td>24.00 mg</td>
</tr>
<tr>
<td>Na₂MoO₄ x 2 H₂O</td>
<td>36.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>990.00 ml</td>
</tr>
</tbody>
</table>

First, dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

### Wolin's vitamin solution (10x) (from medium 120)

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>20.00 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>20.00 mg</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Calcium D-(-)-pantothenate</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1.00 mg</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>(DL)-alpha-Lipoic acid</td>
<td>50.00 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml</td>
</tr>
</tbody>
</table>