

**1501. METHYLOCALDUM MARINUM MEDIUM****Solution 1** (10x NMS salts):

|                                        |         |    |
|----------------------------------------|---------|----|
| KNO <sub>3</sub>                       | 10.00   | g  |
| MgSO <sub>4</sub> x 6 H <sub>2</sub> O | 10.00   | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O | 2.00    | g  |
| Distilled water                        | 1000.00 | ml |

Dissolve the ingredients listed above (in that order) in about 700 ml of 2% NaCl, and then make up to 1 litre with 2% NaCl.

**Solution 2** (Fe EDTA):

|                 |        |    |
|-----------------|--------|----|
| Fe EDTA         | 3.80   | g  |
| Distilled water | 100.00 | ml |

**Solution 3** (Sodium molybdate):

|                                                       |         |    |
|-------------------------------------------------------|---------|----|
| Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O | 0.26    | g  |
| Distilled water                                       | 1000.00 | ml |

*Trace elements:*

|                                        |        |    |
|----------------------------------------|--------|----|
| EDTA di sodium salt                    | 25.00  | mg |
| CuSO <sub>4</sub> x 5 H <sub>2</sub> O | 20.00  | mg |
| FeSO <sub>4</sub> x 7 H <sub>2</sub> O | 50.00  | mg |
| ZnSO <sub>4</sub> x 7 H <sub>2</sub> O | 40.00  | mg |
| H <sub>3</sub> BO <sub>3</sub>         | 1.50   | mg |
| CoCl <sub>2</sub> x 6 H <sub>2</sub> O | 5.00   | mg |
| MnCl <sub>2</sub> x 4 H <sub>2</sub> O | 2.00   | mg |
| NiCl <sub>2</sub> x 6 H <sub>2</sub> O | 1.00   | mg |
| Distilled water                        | 100.00 | ml |

May be stored at 4°C in the dark

*Phosphate buffer:*

|                                                        |         |    |
|--------------------------------------------------------|---------|----|
| Na <sub>2</sub> HPO <sub>4</sub> x 12 H <sub>2</sub> O | 71.60   | g  |
| KH <sub>2</sub> PO <sub>4</sub>                        | 26.00   | g  |
| Distilled water                                        | 1000.00 | ml |

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

*Prepare the growth medium as follows:*

Dilute 100 ml solution 1 to 1 litre with 2% NaCl and then add 1 ml of solution 3, 1 ml of the trace elements, and 0.1 ml of solution 2. If solid media is required add 1.5% agar. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 25% methane to the gas phase and autoclave at 121°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. When the growth medium is cool 10 ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.