1488. METHYLOCEANUS CENITEPIDUS MEDIUM

**Solution 1** (10x NMS salts):

- NH₄Cl: 10.00 g
- MgSO₄ · 6 H₂O: 10.00 g
- CaCl₂ · 2 H₂O: 2.00 g
- 2% NaCl in 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):

- Na₂MoO₄ · 2 H₂O: 0.26 g
- Distilled water: 1000.00 ml

**Trace elements**:

- EDTA di sodium salt: 25.00 mg
- CuSO₄ · 5 H₂O: 20.00 mg
- FeSO₄ · 7 H₂O: 50.00 mg
- ZnSO₄ · 7 H₂O: 40.00 mg
- H₃BO₃: 1.50 mg
- CoCl₂ · 6 H₂O: 5.00 mg
- MnCl₂ · 4 H₂O: 2.00 mg
- NiCl₂ · 6 H₂O: 1.00 mg
- Distilled water: 100.00 ml

May be stored at 4°C in the dark.

**Phosphate buffer**:

- Na₂HPO₄ · 12 H₂O: 71.60 g
- KH₂PO₄: 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. 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. Add filter-sterilized methanol to produce 1% (final concentration).