141. METHANOGENIUM MEDIUM (H₂/CO₂)

KCl 0.34 g  
MgCl₂ × 6 H₂O 4.00 g  
MgSO₄ × 7 H₂O 3.45 g  
NH₄Cl 0.25 g  
CaCl₂ × 2 H₂O 0.14 g  
K₂HPO₄ 0.14 g  
NaCl 18.00 g  
Trace element solution (see below) 10.00 ml  
Fe(NH₄)₂(SO₄)₂ × 6 H₂O solution (0.1% w/v) 2.00 ml  
Na-acetate 1.00 g  
Yeast extract (OXOID) 2.00 g  
Trypticase peptone (BD BBL) 2.00 g  
Na-resazurin solution (0.1% w/v) 0.50 ml  
NaHCO₃ 5.00 g  
Vitamin solution (see below) 10.00 ml  
L-Cysteine-HCl x H₂O 0.50 g  
Na₂S x 9 H₂O 0.50 g  
Distilled water 1000.00 ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 – 7.0, if necessary. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at two atmospheres of pressure.  
*Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.*
**Trace element solution:**

- Nitrilotriacetic acid: 1.50 g
- MgSO₄ x 7 H₂O: 3.00 g
- MnSO₄ x H₂O: 0.50 g
- NaCl: 1.00 g
- FeSO₄ x 7 H₂O: 0.10 g
- CoSO₄ x 7 H₂O: 0.18 g
- CaCl₂ x 2 H₂O: 0.18 g
- ZnSO₄ x 7 H₂O: 0.18 g
- CuSO₄ x 5 H₂O: 0.01 g
- KAl(SO₄)₂ x 12 H₂O: 0.02 g
- H₃BO₃: 0.01 g
- Na₂MoO₄ x 2 H₂O: 0.01 g
- NiCl₂ x 6 H₂O: 0.03 g
- Na₂SeO₃ x 5 H₂O: 0.30 mg
- Na₂WO₄ x 2 H₂O: 0.40 mg
- Distilled water: 1000.00 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

**Vitamin solution:**

- Biotin: 2.00 mg
- Folic acid: 2.00 mg
- Pyridoxine-HCl: 10.00 mg
- Thiamine-HCl x 2 H₂O: 5.00 mg
- Riboflavin: 5.00 mg
- Nicotinic acid: 5.00 mg
- D-Ca-pantothenate: 5.00 mg
- Vitamin B₁₂: 0.10 mg
- p-Aminobenzoic acid: 5.00 mg
- Lipoic acid: 5.00 mg
- Distilled water: 1000.00 ml

For DSM 1498 and DSM 22353 adjust pH to 6.5.

For DSM 2373 increase the amount of trypticase to 6.00 g/l.

For DSM 4254 add a filter-sterilized, anoxic solution of L-histidine to a final concentration of 80.00 mg/l.

*Continued on next page*
For DSM 7268, DSM 7466 and DSM 14266 use only one atmosphere overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.

For DSM 15219, DSM 18860 and DSM 21220 adjust pH to 7.5.

For DSM 15558 supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptopethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N₂ gas. Adjust pH to 6.5 and use only one atmosphere overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.

For DSM 16458 supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptopethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N₂ gas. Adjust pH to 7.5 and use only one atmosphere overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.