

141: METHANOGENIUM MEDIUM (H₂/CO₂)

This recipe contains strain-specific modifications for *Methanoculleus marisnigri* DSM 1498 *

Final pH: * 6.5

Final volume: 1013 ml

| | | |
|---|---------|----|
| KCl | 0.34 | g |
| MgCl ₂ x 6 H ₂ O | 4.00 | g |
| MgSO ₄ x 7 H ₂ O | 3.45 | g |
| NH ₄ Cl | 0.25 | g |
| CaCl ₂ x 2 H ₂ O | 0.14 | g |
| K ₂ HPO ₄ | 0.14 | g |
| NaCl | 18.00 | g |
| Modified Wolin's mineral solution | 10.00 | ml |
| Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (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 |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| Wolin's vitamin solution | 10.00 | ml |
| NaHCO ₃ | 5.00 | g |
| L-Cysteine HCl x H ₂ O | 0.50 | g |
| Na ₂ S x 9 H ₂ O | 0.50 | g |
| Distilled water | 1000.00 | ml |

1. Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.

2. 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 6.5, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume 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.

3. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at two atmospheres of pressure.

* Adjust pH to 6.5.

Modified Wolin's mineral solution (from medium 141)

| | | |
|-----------------------|------|---|
| Nitrilotriacetic acid | 1.50 | g |
|-----------------------|------|---|

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| | | |
|--|---------|----|
| MgSO ₄ x 7 H ₂ O | 3.00 | g |
| MnSO ₄ x H ₂ O | 0.50 | g |
| NaCl | 1.00 | g |
| FeSO ₄ x 7 H ₂ O | 0.10 | g |
| CoSO ₄ x 7 H ₂ O | 0.18 | g |
| CaCl ₂ x 2 H ₂ O | 0.10 | g |
| ZnSO ₄ x 7 H ₂ O | 0.18 | g |
| CuSO ₄ x 5 H ₂ O | 0.01 | g |
| AlK(SO ₄) ₂ x 12 H ₂ O | 0.02 | g |
| H ₃ BO ₃ | 0.01 | g |
| Na ₂ MoO ₄ x 2 H ₂ O | 0.01 | g |
| NiCl ₂ x 6 H ₂ O | 0.03 | g |
| Na ₂ SeO ₃ x 5 H ₂ O | 0.30 | mg |
| Na ₂ WO ₄ x 2 H ₂ O | 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.

Wolin's vitamin solution (from medium 141)

| | | |
|----------------------------|---------|----|
| Biotin | 2.00 | mg |
| Folic acid | 2.00 | mg |
| Pyridoxine hydrochloride | 10.00 | mg |
| Thiamine HCl | 5.00 | mg |
| Riboflavin | 5.00 | mg |
| Nicotinic acid | 5.00 | mg |
| Calcium D-(+)-pantothenate | 5.00 | mg |
| Vitamin B ₁₂ | 0.10 | mg |
| p-Aminobenzoic acid | 5.00 | mg |
| (DL)-alpha-Lipoic acid | 5.00 | mg |
| Distilled water | 1000.00 | ml |