

342: METHANOBACTERIUM ALCALIPHILUM MEDIUM

Solution A	792.00	ml
Solution B	210.00	ml
Solution C	10.00	ml

1. Dissolve ingredients of solution A (except cysteine), bring to the boil, then cool to room temperature under 100% H₂ gas. Add cysteine and adjust pH to 7.0, if necessary. Dispense medium under 100% H₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and then autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas atmosphere. Complete the medium by adding appropriate amounts of solutions B and C to the sterile solution A. Adjust pH of complete medium to 8.3 - 8.4, if necessary.

2. After inoculation add sterile H₂ gas to 1 - 2 bar overpressure.

Solution A

NH ₄ Cl	1.00	g
MgCl ₂ x 6 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.10	g
K ₂ HPO ₄	0.40	g
Na ₂ SeO ₃ x 5 H ₂ O (0.01% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	780.00	ml

Solution B

NaHCO ₃	10.00	g
Na ₂ CO ₃	0.50	g
Distilled water	210.00	ml

Solution C

Tris-HCl buffer (pH 8.4, 2 M)	10.00	ml
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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g

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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.