

274: METHANOLACINIA MEDIUM

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	2.75	g
MgSO ₄ x 7 H ₂ O	3.40	g
NH ₄ Cl	1.50	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	6.31	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
NaHCO ₃	5.00	g
Na-acetate x 3 H ₂ O	4.15	g
Sodium resazurin (0.1% w/v)	0.50	ml
MOPS (3-(N-morpholino) propane-sulfonic acid)	20.93	g
Wolin's vitamin solution (10x)	1.00	ml
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. Dissolve ingredients except MOPS, vitamins, cysteine and sulfide in a total volume of 890 ml. The MOPS buffer is dissolved separately in 90 ml of distilled water, the pH is adjusted to 7.0 with 2 M KOH, then add the buffer to the salt solution. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then dispense medium in anoxic Hungate-type tubes under the same gas atmosphere to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Prior to use check pH of complete medium and adjust to 7.0, if necessary.

2. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure.

Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
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 (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml