

404: METHANOSPHERA CUNICULI MEDIUM

Mineral solution 1	40.00	ml
Minerals solution	40.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 7 H ₂ O (0.1% w/v)	2.00	ml
NiCl ₂ x 6 H ₂ O (0.1% w/v)	0.20	ml
Modified Wolin's mineral solution	10.00	ml
Na-acetate x 3 H ₂ O	3.00	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	3.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	900.00	ml

1. Dissolve ingredients (except bicarbonate, methanol, vitamins, cysteine and sulfide), then 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 distribute 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 methanol (50% v/v), vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2.

2. After inoculation, pressurize culture bottles with sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.

Mineral solution 1 (from medium 203)

K ₂ HPO ₄	6.00	g
Distilled water	1000.00	ml

Minerals solution (from medium 317)

KH ₂ PO ₄	6.00	g
(NH ₄) ₂ SO ₄	6.00	g
NaCl	12.00	g
MgSO ₄ x 7 H ₂ O	2.60	g
CaCl ₂ x 2 H ₂ O	0.16	g
Distilled water	1000.00	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
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