

282: METHANOCALDOCOCCLUS MEDIUM

Final pH: 6.0

Final volume: 1012 ml

K ₂ HPO ₄	0.14	g
CaCl ₂ x 2 H ₂ O	0.14	g
NH ₄ Cl	0.25	g
MgSO ₄ x 7 H ₂ O	3.40	g
MgCl ₂ x 6 H ₂ O	4.10	g
KCl	0.33	g
NiCl ₂ x 6 H ₂ O (0.1% w/v)	0.50	ml
NaCl	30.00	g
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O	0.01	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	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 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, then dispense medium into anoxic tubes to 30% of their volume under 80% H₂ and 20% CO₂ gas atmosphere 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 completed medium and adjust to 6.0, if necessary.

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

For [DSM 5666](#), [DSM 111364](#): Adjust pH of complete medium to 6.5.

For [DSM 16983](#), [DSM 27223](#): Adjust pH of complete medium to 7.0 - 7.2.

For [DSM 26965](#): Supplement medium with 3.00 g/l yeast extract and add 2.50 g/l maltose to the autoclaved medium from a sterile anoxic stock solution prepared under 100% N₂ gas. Adjust pH of complete medium to 6.5 and use without overpressure of 80% H₂ and 20% CO₂ gas mixture.

For [DSM 100453](#): Supplement medium with 1 g/l Na₂S₂O₃ x 5 H₂O, added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

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 (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