

203: METHANOTHERMUS MEDIUM

Mineral solution 1	37.50	ml
Mineral solution 2	37.50	ml
NiCl ₂ x 6 H ₂ O (0.1% w/v)	1.00	ml
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Na ₂ SO ₄	3.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	920.00	ml

1. Dissolve ingredients except carbonate, yeast extract, Trypticase peptone, vitamins, sulfide and cysteine, then adjust pH to 6.0 with sulfuric acid. Sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum bottles to 30% of their volume and autoclave. Thereafter, add by injection yeast extract, Trypticase peptone, vitamins (sterilized by filtration), sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 6.5.

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

For DSM 3496: Omit yeast extract and Trypticase peptone from the medium.

Mineral solution 1 (from medium 203)

K ₂ HPO ₄	6.00	g
Distilled water	1000.00	ml

Mineral solution 2 (from medium 203)

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	2.40	g
CaCl ₂ x 2 H ₂ O	1.60	g
Distilled water	1000.00	ml

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