

## 203: METHANOTHERMUS MEDIUM

<b>Mineral solution 1</b>	37.50	ml
<b>Mineral solution 2</b>	37.50	ml
NiCl <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	1.00	ml
FeSO <sub>4</sub> x 7 H <sub>2</sub> O (0.1% w/v in 0.1 N H <sub>2</sub> SO <sub>4</sub> )	2.00	ml
<b>Modified Wolin's mineral solution</b>	10.00	ml
Na <sub>2</sub> SO <sub>4</sub>	3.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
L-Cysteine HCl x H <sub>2</sub> 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% H<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of complete medium to 6.5.

2. After inoculation pressurize bottles to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

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

### Mineral solution 1 (from medium 203)

K <sub>2</sub> HPO <sub>4</sub>	6.00	g
Distilled water	1000.00	ml

### Mineral solution 2 (from medium 203)

KH <sub>2</sub> PO <sub>4</sub>	6.00	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	6.00	g
NaCl	12.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	2.40	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.60	g
Distilled water	1000.00	ml

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### Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml