

## 141. METHANOGENIUM MEDIUM (H<sub>2</sub>/CO<sub>2</sub>)

KCl	0.34	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.45	g
NH <sub>4</sub> Cl	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
NaCl	18.00	g
Trace element solution (see below)	10.00	ml
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O solution (0.1% w/v)	2.00	ml
Na-acetate	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	5.00	g
Vitamin solution (see below)	10.00	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense medium under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N<sub>2</sub> gas. Vitamins are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 – 7.0, if necessary. For incubation use sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture at two atmospheres of pressure.

*Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.*

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*Trace element solution:*

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
KAl(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.

*Vitamin solution:*

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B <sub>12</sub>	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml

For [DSM 1498](#) and [DSM 22353](#) adjust pH to 6.5.

For [DSM 2373](#) increase the amount of trypticase to 6.00 g/l.

For [DSM 4254](#) add a filter-sterilized, anoxic solution of L-histidine to a final concentration of 80.00 mg/l.

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For DSM 7268, DSM 7466 and DSM 14266 use only one atmosphere overpressure of sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

For DSM 15219, DSM 18860 and DSM 21220 adjust pH to 7.5.

For DSM 15558 supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N<sub>2</sub> gas. Adjust pH to 6.5 and use only one atmosphere overpressure of sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

For DSM 16458 supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under 100% N<sub>2</sub> gas. Adjust pH to 7.5 and use only one atmosphere overpressure of sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.