

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

This recipe contains strain-specific modifications for *Methanosphaera cuniculi* DSM 4103 \*

Final pH: 6.8 - 7.0

Final volume: 1013 ml

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
<b>Modified Wolin's mineral solution</b>	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 (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
Sodium resazurin (0.1% w/v)	0.50	ml
<b>Wolin's vitamin solution</b>	10.00	ml
NaHCO <sub>3</sub>	5.00	g
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
<b>Methanol</b>	<b>5.00</b>	<b>ml</b>
Distilled water	1000.00	ml

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

2. 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 6.5, 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.

3. For incubation use sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture at two atmospheres of pressure.

\* Supplement medium with 10 ml of a methanol solution (50% v/v) added to the autoclaved medium from a sterile anoxic stock solution.

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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 (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B <sub>12</sub>	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml