

## 404. METHANOSPHERA CUNICULI MEDIUM

Mineral solution 1 (see below)	40.00	ml
Mineral solution 2 (see below)	40.00	ml
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 7 H <sub>2</sub> O solution (0.1% w/v)	2.00	ml
NiCl <sub>2</sub> x 6 H <sub>2</sub> O solution (0.1% w/v)	0.20	ml
Trace element solution (see medium 141)	10.00	ml
Na-acetate x 3 H <sub>2</sub> O	3.00	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	3.00	g
Methanol	5.00	ml
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	900.00	ml

Dissolve ingredients (except bicarbonate, methanol, vitamins, cysteine and sulfide), then 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 distribute under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add methanol, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 7.0 – 7.2. After inoculation, pressurize culture bottles with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.

### *Mineral solution 1:*

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

### *Mineral solution 2:*

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	2.60	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.16	g
Distilled water	1000.00	ml