

404. METHANOSPHERA CUNICULI MEDIUM

Mineral solution 1 (see below)	40.00	ml
Mineral solution 2 (see below)	40.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 7 H ₂ O solution (0.1% w/v)	2.00	ml
NiCl ₂ x 6 H ₂ O solution (0.1% w/v)	0.20	ml
Trace element solution (see medium 141)	10.00	ml
Na-acetate x 3 H ₂ 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 ₃	3.00	g
Methanol	5.00	ml
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	900.00	ml

Dissolve ingredients (except bicarbonate, methanol, vitamins, cysteine and sulfide), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then distribute under 80% H₂ and 20% CO₂ 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% N₂ 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% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.

Mineral solution 1:

K ₂ HPO ₄	6.00	g
Distilled water	1000.00	ml

Mineral solution 2:

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	2.60	g
CaCl ₂ x 2 H ₂ O	0.16	g
Distilled water	1000.00	ml