

373. METHANOGENIUM CV MEDIUM

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	4.00	g
MgSO ₄ x 7 H ₂ O	3.45	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	18.00	g
Trace element solution (see medium 141)	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ 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 ₃	5.00	g
Isopropyl alcohol	7.50	ml
Vitamin solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, isopropyl alcohol, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add isopropyl alcohol and cysteine from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.8 - 7.0, if necessary.