

## 373. METHANOGENIUM CV MEDIUM

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 medium 141)	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
Isopropyl alcohol	7.50	ml
Vitamin solution (see medium 141)	10.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, isopropyl alcohol, vitamins and sulfide), then sparge medium with 80% N<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 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% N<sub>2</sub> gas atmosphere. 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.