

**141a. METHANOCOCCUS SP. 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
Na-resazurin solution (0.1% w/v)	0.50	ml
Vitamin solution (see medium 141)	10.00	ml
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
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, cysteine and sulfide), sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to reach a pH below 6 (at least 30 min), then dispense medium under same 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 atmosphere. Vitamins are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. For incubation use sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture at two atmospheres of pressure. Adjust pH of final medium to 5.5 – 5.7.