

**282. METHANOCALDOCOCCLUS MEDIUM**

K <sub>2</sub> HPO <sub>4</sub>	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
NH <sub>4</sub> Cl	0.25	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.10	g
KCl	0.33	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O solution (0.1% w/v)	0.50	ml
NaCl	30.00	g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O	0.01	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	1.00	g
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 bicarbonate, vitamins, cysteine and sulfide. 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, then dispense medium into anoxic tubes under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Prior to use check pH of completed medium and adjust to 6.0, if necessary. After inoculation add sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.

For [DSM 5666](#) adjust pH of complete medium to 6.5.

For [DSM 16983](#) and [DSM 27223](#) adjust pH of complete medium to 7.0 – 7.2.

For [DSM 26965](#) supplement medium with 3.00 g/l yeast extract and add 2.50 g/l maltose to the autoclaved medium from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas. Adjust pH of complete medium to 6.5 and use without overpressure of 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.