

**ATCC medium: 1892 *Methanobacterium* medium (DSM 119)**

KH <sub>2</sub> PO <sub>4</sub> .....	0.5 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O.....	0.4 g
NaCl.....	0.4 g
NH <sub>4</sub> Cl.....	0.4 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O.....	50.0 mg
FeSO <sub>4</sub> . 7H <sub>2</sub> O.....	2.0 mg
Trace Elements Solution SL-10 (see below).....	1.0 ml
Yeast extract.....	1.0 g
Sodium acetate.....	1.0 g
Sodium formate.....	2.0 g
Sludge Fluid (see below).....	50.0 ml
NaHCO <sub>3</sub> .....	4.0 g
Fatty Acid Mixture (see below).....	20.0 ml
Resazurin.....	1.0 mg
L-Cysteine . HCl . H <sub>2</sub> O.....	0.5 g
Na <sub>2</sub> S . 9H <sub>2</sub> O.....	0.5 g
Distilled water.....	940.0 ml

Combine components except sodium bicarbonate and reducing agents. Bring to boiling, then cool to room temperature under an atmosphere of 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Add sodium bicarbonate and equilibrate under same gas phase for 5-10 minutes. Add cysteine and sodium sulfide. If necessary, adjust for final pH 6.7-7.0. Dispense medium anaerobically under a gas atmosphere of 80% H<sub>2</sub>, 20% CO<sub>2</sub>. Autoclave at 121C for 15 minutes.

*Trace Elements Solution SL-10:*

HCl (25%).....	10.0 ml
FeCl <sub>2</sub> . 4H <sub>2</sub> O.....	1.5 g
ZnCl <sub>2</sub> .....	70.0 mg
MnCl <sub>2</sub> . 4H <sub>2</sub> O.....	100.0 mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0 mg
CoCl <sub>2</sub> . 6H <sub>2</sub> O.....	190.0 mg
CuCl <sub>2</sub> . 2H <sub>2</sub> O.....	2.0 mg
NiCl <sub>2</sub> . 6H <sub>2</sub> O.....	24.0 mg
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	36.0 mg
Distilled water.....	990.0 ml

Dissolve FeCl<sub>2</sub> in the HCl, dilute with water, add and dissolve the other salts, adjust pH to 6.0 with NaOH, and fill up to 1.0 L.

*Sludge Fluid:*

Add 0.4 % yeast extract to sludge from an anaerobic digester and, after gassing with N<sub>2</sub> for a few minutes, incubate it at 37C for 24 hours. Centrifuge the sludge at 13,000 X g; bottle and autoclave the resulting clear supernatant under N<sub>2</sub>. Store the processed sludge fluid at room temperature in the dark.

*Fatty Acid Mixture:*

Valeric acid.....	0.5 g
Isovaleric acid.....	0.5 g
alpha-Methylbutyric acid.....	0.5 g
Isobutyric acid.....	0.5 g
Distilled water.....	20.0 ml

Adjust fatty acid mixture to pH 7.5 with concentrated NaOH.