

ATCC medium: 1471 Sludge medium for *Methanobacteria*

KH ₂ PO ₄	0.5 g
MgSO ₄ . 7H ₂ O	0.4 g
NaCl.....	0.4 g
NH ₄ Cl	0.4 g
CaCl ₂ . 2H ₂ O	0.05 g
FeSO ₄ . 7H ₂ O	2.0 mg
Trace Elements Solution SL-6 (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 ₃	4.0 g
Fatty Acid Mixture (see below).....	20.0 ml
Resazurin.....	1.0 mg
L-Cysteine HCl.....	0.5 g
Na ₂ S . 9H ₂ O	0.5 g
Distilled water.....	940.0 ml

Adjust pH to 6.7-7.0. Prepare and dispense medium anaerobically under 80% N₂, 20% CO₂ into aluminum seal-type culture tubes. Autoclave at 121C for 15 minutes under fast exhaust.

Trace Elements Solution SL-6:

ZnSO ₄ . 7H ₂ O	0.10 g
MnCl ₂ . 4H ₂ O	0.03 g
H ₃ BO ₃	0.3 g
CoCl ₂ . 6H ₂ O	0.2 g
CuCl ₂ . 2H ₂ O	0.01 g
NiCl ₂ . 6H ₂ O	0.02 g
Na ₂ MoO ₄ . 2H ₂ O.....	0.03 g
Distilled water.....	1.0 L

Adjust final pH of Trace Elements Solution SL-6 to 3.4.

Sludge Fluid:

To sludge from an anaerobic digester, add yeast extract to a concentration of 0.4% and after gassing with nitrogen gas for a few minutes incubate at 37C for 24 hours. Centrifuge at 13000 g and remove the supernate. Autoclave the supernate in screw-capped bottles under nitrogen gas. The sludge fluid can be stored 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 pH to 7.5 with concentrated NaOH.