

ATCC medium: 1043 *Methanosarcina* medium

K ₂ HPO ₄	348.0 mg
KH ₂ PO ₄	227.0 mg
NH ₄ Cl	500.0 mg
MgSO ₄ . 7H ₂ O	500.0 mg
CaCl ₂ . 2H ₂ O	250.0 mg
FeSO ₄ . 7H ₂ O	2.0 mg
Trace Elements Solution SL-6 (see below)...	3.0 ml
Wolfe's Vitamin Solution (see below).....	10.0 ml
Yeast Extract (BD 212750).....	2.0 g
Casitone (BD 225930).....	2.0 g
NaCl.....	2.25 g
Resazurin (0.025% solution).....	4.0 ml
NaHCO ₃ Solution (see below)	20.0 ml
Methanol.....	10.0 ml
Reducing Agent (see below).....	20.0 ml
Agar.....	20.0 g
Distilled water.....	950.0 ml

Mix all of the ingredients except the Wolfe's Vitamin Solution, NaHCO₃ Solution, Methanol and Cysteine/Na₂S Solution (Reducing Agent) and autoclave for 15 minutes. After autoclaving, cool under 80% N₂, 20% CO₂ and add the solutions in the order listed above. Adjust pH to 6.8 if necessary and tube anaerobically and aseptically.

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

Wolfe's Vitamin Solution:

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Biotin.....	2.0 mg
Folic acid.....	2.0 mg
Pyridoxine hydrochloride.....	10.0 mg
Thiamine . HCl.....	5.0 mg
Riboflavin.....	5.0 mg
Nicotinic acid.....	5.0 mg
Calcium D-(+)-pantothenate.....	5.0 mg
Vitamin B12.....	0.1 mg
p-Aminobenzoic acid.....	5.0 mg
Thioctic acid.....	5.0 mg
Distilled water.....	1.0 L

NaHCO₃ Solution:

Dissolve 850 mg of NaHCO₃ in 20.0 ml of water. After filter-sterilizing, equilibrate under CO₂ for 20 minutes.

Reducing Agent:

Add 300 mg L-cysteine . HCl to 10 ml of water and 300 mg Na₂S . 9H₂O to a second 10 ml of water; tube under nitrogen gas. After autoclaving at 121C for 15 minutes under fast exhaust and allowing to cool, mix equal amounts of the cysteine and the sodium sulfide (under nitrogen). The mixture should be clear and can be used up to two weeks.