

ATCC medium: 2283 *Methanomicrococcus* medium

K ₂ HPO ₄	0.348 g
KH ₂ PO ₄	0.227 g
NH ₄ Cl	0.5 g
MgSO ₄ . 7H ₂ O	0.5 g
CaCl ₂ . 2H ₂ O	0.25 g
NaCl.....	2.25 g
Sodium acetate.....	2.5 g
Coenzyme M*.....	10.0 mg
FeSO ₄ . 7H ₂ O	0.002 g
Wolfe's Vitamin Solution (see below)	10.0 ml
Trace Elements Solution SL-10 (see below)	1.0 ml
Yeast extract.....	2.0 g
Casitone.....	2.0 g
Resazurin.....	0.001 g
NaHCO ₃	2.85 g
Methanol.....	20.0 ml
L-Cysteine . HCl.....	0.75 g
Na ₂ S . 9H ₂ O	0.75 g
Distilled water.....	1.0 L

*Coenzyme M = 2-Mercaptoethanesulfonate, Sodium salt (Sigma M-1511, or equiv.)

Prepare the medium anaerobically under an atmosphere of 80% N₂, 20% CO₂. Boil base medium and autoclave at 121C for 15 minutes. Allow to cool under gas. Methanol, cysteine, and sulfide may be prepared as concentrated solutions and autoclaved in tightly capped tubes. Add appropriate volumes of the solutions to the autoclaved base medium. Adjust final pH to 7.2 +/- 0.1. Dispense anaerobically.

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

Trace Elements Solution SL-10:

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

Dissolve FeCl₂ in the HCl, then dilute with water, add and dissolve the other salts, adjust pH to 6.0 with NaOH, finally fill to 1.0 L with distilled water.