

**ATCC medium: 1442 *Methanoculleus thermophilus* medium**

MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	4.0 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.075 g
NaCl.....	11.7 g
K <sub>2</sub> HPO <sub>4</sub> .....	0.14 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.14 g
NaHCO <sub>3</sub> .....	4.0 g
Yeast Extract (BD 212750).....	2.0 g
Trypticase Peptone (BD 211921).....	6.0 g
Resazurin.....	1.0 mg
L-Cysteine . HCl.....	0.5 g
Na <sub>2</sub> S.....	0.25 g
Wolfe's Vitamin Solution (see below)....	10.0 ml
Sodium formate.....	5.0 g
Distilled water to.....	1.0 L

Add all constituents except cysteine and sodium sulfide to the distilled water. Bubble the solution with oxygen-free N<sub>2</sub> and CO<sub>2</sub> (80-20%) gas for 20 minutes. Adjust the pH to 7.2 with 2 M KOH and add the cysteine and sodium sulfide. Tube the medium under N<sub>2</sub> and CO<sub>2</sub> (80-20%) in either serum tubes or bottles. After sterilization the medium may precipitate out; allow it to cool and mix to bring the precipitate back into solution.

*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