

**ATCC medium: 2637 Gracilibacter thermotolerans Medium**

NaH <sub>2</sub> PO <sub>4</sub> .....	0.25 g
NaHCO <sub>3</sub> .....	2.55 g
NaSO <sub>4</sub> .....	1.20 g
NH <sub>4</sub> Cl .....	0.25 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.04 g
CaCl <sub>2</sub> .....	0.01 g
NaCl .....	1.00 g
Yeast extract .....	0.50 g
Wolfe's Mineral Solution (see below) .....	1.00 ml
Wolfe's Vitamin Solution (see below) .....	1.00 ml
Resazurin .....	0.50 mg
Cysteine-HCl. 5H <sub>2</sub> O .....	0.30 g
Distilled water .....	1.0 L

Prepare the medium anaerobically under a steady stream of 80% N<sub>2</sub> and 20% CO<sub>2</sub>. The pH of the medium should be adjusted to 7.2. Autoclave at 121°C for 15 minutes. Filter sterilize and prepare separately an anaerobic 2M glucose stock solution. Add 100ul of the glucose stock solution for every 10ml of media.

*Wolfe's Mineral Solution:*

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid .....	1.500 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	3.000 g
MnSO <sub>4</sub> . H <sub>2</sub> O .....	0.500 g
NaCl .....	1.000 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.100 g
Co(NO <sub>3</sub> ) <sub>2</sub> . 6H <sub>2</sub> O .....	0.100 g
CaCl <sub>2</sub> .....	0.100 g
ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.100 g
CuSO <sub>4</sub> . 5H <sub>2</sub> O .....	0.010 g
AlK(SO <sub>4</sub> ) <sub>2</sub> . 12H <sub>2</sub> O .....	0.010 g
H <sub>3</sub> BO <sub>3</sub> .....	0.010 g
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O .....	0.010 g
Na <sub>2</sub> SeO <sub>3</sub> (anhydrous) .....	0.001 g
Na <sub>2</sub> WO <sub>4</sub> . 2H <sub>2</sub> O .....	0.010 g
NiCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.020 g
Distilled water .....	1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

*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