

**ATCC medium: 2636 Thermalkalibacillus uzonensis Medium**

NH<sub>4</sub>Cl .....1.00 g  
NaCl.....3.60 g  
KCl.....1.00 g  
Na<sub>2</sub>SO<sub>4</sub> .....0.50 g  
CaCl<sub>2</sub> .....0.04 g  
MgCl<sub>2</sub> . 6H<sub>2</sub>O .....0.04 g  
KH<sub>2</sub>PO<sub>4</sub> .....0.50 g  
K<sub>2</sub>HPO<sub>4</sub> .....1.00 g  
Wolfe's Mineral Solution (see below).....1.00 ml  
Wolfe's Vitamin Solution (see below).....1.00 ml  
Yeast extract.....0.50 g  
Distilled water.....1.0 L

The pH of the medium should be adjusted to 8.3. Autoclave at 121°C for 15 minutes. Filter sterilize and prepare separately a 2M pyruvate stock solution. Add 100ul of the pyruvate 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