

**ATCC medium: 1613 *Clostridium thermolacticum* medium**

K <sub>2</sub> HPO <sub>4</sub> .....	0.348 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.227 g
NH <sub>4</sub> Cl .....	0.5 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.5 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.25 g
NaCl.....	2.25 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	2.0 mg
Wolfe's Vitamin Solution (see below)....	10.0 ml
Trace Element Solution SL-6 (see below)..	3.0 ml
Yeast Extract (BD 212750).....	2.0 g
Resazurin.....	1.0 mg
KHCO <sub>3</sub> .....	4.5 g
Sucrose.....	2.0 g
L-Cysteine . HCl.....	0.3 g
Na <sub>2</sub> S . 9H <sub>2</sub> O .....	0.3 g
Distilled water.....	1.0 L

Prepare the medium anaerobically under an atmosphere of 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Autoclave each reducing agent separately under N<sub>2</sub> atmosphere as a concentrated solution in tightly closed tubes. Inject appropriate volumes of the solutions into the autoclaved main part of medium with hypodermic syringes. Final pH 7.0-7.2.

*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-6:*

ZnSO<sub>4</sub> · 7H<sub>2</sub>O .....0.10 g  
MnCl<sub>2</sub> · 4H<sub>2</sub>O .....0.03 g  
H<sub>3</sub>BO<sub>3</sub> .....0.3 g  
CoCl<sub>2</sub> · 6H<sub>2</sub>O .....0.2 g  
CuCl<sub>2</sub> · 2H<sub>2</sub>O .....0.01 g  
NiCl<sub>2</sub> · 6H<sub>2</sub>O .....0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O.....0.03 g  
Distilled water.....1.0 L

Adjust final pH of Trace Elements Solution SL-6 to 3.4.