

**ATCC medium: 2677 Pelobacter Medium**

KCl.....	1.30 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.20 g
NaCl.....	23.00 g
NH <sub>4</sub> Cl .....	0.50 g
CaCl <sub>2</sub> * 2H <sub>2</sub> O .....	0.10 g
MgCl <sub>2</sub> * 6H <sub>2</sub> O.....	3.00 g
NaHCO <sub>3</sub> .....	2.50 g
Wolfe's Vitamin solution (see Below).....	5.00 ml
Trace salt I (see Below).....	1.00 ml
Trace salt II (see Below).....	0.10 ml
Resazurin (1g/L stock).....	0.20 ml
Lactate (1M stock).....	10.00 mM
Na <sub>2</sub> S . 9H <sub>2</sub> O (500g/L stock).....	0.20 ml
Distilled water.....	1.00 L

*Combine the first six ingredients in 1.0L of DI water. Place the medium under a steady stream of N<sub>2</sub> for 30 to 45 minutes. Continue to purge with N<sub>2</sub> and add the Sodium bicarbonate (NaHCO<sub>3</sub>). Seal the bottle and autoclave for 15 minutes at 121°C for 15 minutes. After cooling to room temperature aseptically add the anoxic sterile stock solution Trace Salt I and II, Vitamin solution, Resazurin, Lactate (stock 1M) and sodium sulfide. Check the pH and adjust to 7.0.*

**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

*Filter sterilize and store as a stock solution*

**Trace Salt I**

MnCl<sub>2</sub> · 6H<sub>2</sub>O .....5.00 g  
H<sub>3</sub>BO<sub>3</sub> .....0.50 g  
ZnCl<sub>2</sub> .....0.50 g  
CoCl<sub>2</sub> · 6H<sub>2</sub>O.....0.50 g  
NiCl<sub>2</sub> · 6H<sub>2</sub>O .....0.46 g  
CuCl · 2H<sub>2</sub>O .....0.30 g  
Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O.....0.10 g  
FeCl<sub>2</sub> · 4H<sub>2</sub>O .....1.46 g  
Distilled water.....1.00 L

*Filter sterilize and store as a stock solution*

**Trace Salt II**

Na<sub>2</sub>SeO<sub>3</sub> (anhydrous) .....0.03 g  
Na<sub>2</sub>WO<sub>4</sub> · 2H<sub>2</sub>O.....0.08 g  
Distilled water.....1.00 L

*Filter sterilize and store as a stock solution*