

**ATCC medium: 2035 *Desulfitobacterium chlororespirans* medium**

NaCl.....	1.0 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.5 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2 g
NH <sub>4</sub> Cl .....	0.3 g
KCl.....	0.3 g
CaCl <sub>2</sub> .....	15.0 mg
Modified Wolfe's Minerals (see below).....	10.0 ml
0.1% Resazurin.....	0.5 ml
NaHCO <sub>3</sub> .....	2.52 g
L-Cysteine . HCl.....	35.0 mg
Na <sub>2</sub> S . 9H <sub>2</sub> O .....	48.0 mg
Wolfe's Vitamin Solution (see below).....	10.0 ml
Sodium pyruvate.....	2.2 g
3-Chloro-4-hydroxybenzoic acid . 2H <sub>2</sub> O.....	0.36 g
Distilled water to.....	1.0 L

Add and dissolve components through resazurin to distilled water. Boil and cool under an atmosphere of 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Add sodium bicarbonate and equilibrate for 5 minutes under the same gas phase. Add cysteine and then sodium sulfide and allow medium to reduce. Aliquot medium as desired using anaerobic techniques and closures. Autoclave at 121C for 15 minutes. Just prior to inoculation, add vitamins and sodium pyruvate to the final concentration indicated above from filter-sterilized stock solutions equilibrated under 100% N<sub>2</sub>. Add 3-chloro-4-hydroxybenzoate to the final concentration indicated above from a neutralized stock solution autoclaved under 100% N<sub>2</sub>. Final pH 7.2 - 7.3.

*Modified Wolfe's Minerals:*

Na <sub>2</sub> SeO <sub>3</sub> .....	10.0 mg
NiCl <sub>2</sub> . 6H <sub>2</sub> O .....	10.0 mg <sub>2</sub>
Na <sub>2</sub> WO <sub>4</sub> . 2H <sub>2</sub> O.....	10.0 mg
Wolfe's Mineral Solution (see below).....	1.0 L

*Wolfe's Mineral Solution:*

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

Nitrilotriacetic acid.....	1.5 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	3.0 g
MnSO <sub>4</sub> . H <sub>2</sub> O .....	0.5 g
NaCl.....	1.0 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.1 g
CoCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.1 g
CaCl <sub>2</sub> .....	0.1 g
ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.1 g
CuSO <sub>4</sub> . 5H <sub>2</sub> O .....	0.01 g
AlK(SO <sub>4</sub> ) <sub>2</sub> . 12H <sub>2</sub> O.....	0.01 g
H <sub>3</sub> BO <sub>3</sub> .....	0.01 g
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	0.01 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