

**ATCC medium: 2180 *Orenia salinaria* medium**

NaCl.....150.0 g  
MgCl<sub>2</sub> . 6H<sub>2</sub>O .....15.0 g  
KCl.....3.0 g  
NH<sub>4</sub>Cl .....0.5 g  
KH<sub>2</sub>PO<sub>4</sub> .....0.35 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....0.05 g  
Yeast extract.....0.10 g  
Resazurin (1% solution).....1.0 ml  
Modified Wolfe's Minerals (see below)....1.0 ml  
NaHCO<sub>3</sub>.....2.0 g  
Na<sub>2</sub>S . 9H<sub>2</sub>O .....0.5 g  
Wolfe's Vitamin Solution (see below).....1.0 ml  
Distilled water.....1.0 L

Combine all ingredients except Na<sub>2</sub>S. Bring to a boil for 5 minutes under N<sub>2</sub> gas. Cool under gas and add Na<sub>2</sub>S. Adjust pH to 7.2-7.4. Dispense under gas into aluminum seal tubes. Autoclave at 121C for 15 minutes.

If desired by bacteriologist, carbohydrates (glucose, fructose, and trehalose) should be prepared under gas and filter-sterilized in 10% solutions. They should be added just before inoculation.

*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  
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