

**ATCC medium: 2331 *Roseospira* medium**

*Basal Medium:*

K<sub>2</sub>HPO<sub>4</sub> .....0.3 g  
NH<sub>4</sub>Cl .....0.5 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....0.05 g  
MgCl<sub>2</sub> . 6H<sub>2</sub>O .....1.0 g  
NaCl.....20.0 g  
Wolfe's Mineral Solution (see below).....1.0 ml  
Yeast extract..... 0.5 g  
Distilled deionized water.....1.0 L

Bring to a boil. Autoclave at 121C for 15 minutes.  
Cool under nitrogen gas.

Add 1.0 ml of Solution A.  
Add 10.0 ml of Solution B.  
Add 10.0 ml of Solution C.  
Adjust pH to 6.8 - 7.0.  
Dispense as desired under nitrogen gas.

*Wolfe's Mineral Solution:*

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.

*Solution A (Wolfe's Vitamin Solution):*

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.

*Solution B:*

Sodium ascorbate..... 1.0 g  
Distilled deionized water.....20.0 ml

Filter-sterilize.

*Solution C:*

NaHCO<sub>3</sub>.....3.0 g  
Distilled deionized water.....20.0 ml

Filter-sterilize.