

**ATCC medium: 964 Winogradsky's N-free medium**

Use 5 ml of concentrated salt solution per liter of medium.

Organic matter (sucrose or glucose).....10.0 g/L  
Agar.....20.0 g/L  
CaCO<sub>3</sub>.....trace  
Concentrated Salt Solution (see below)...5.0 ml/L  
Distilled deionized water.....1.0 L

Adjust pH of medium to 7.2. Sterilize at 15 lbs. for 15 minutes.  
Autoclave sugar separately at 10 lbs. for 10 minutes. Add to medium aseptically.

*Concentrated Salt Solution:*

KH<sub>2</sub>PO<sub>4</sub> .....50.0 g  
MgSO<sub>4</sub> . 7H<sub>2</sub>O .....25.0 g  
NaCl.....25.0 g  
FeSO<sub>4</sub> . 7H<sub>2</sub>O .....1.0 g  
MnSO<sub>4</sub> . 4H<sub>2</sub>O .....1.0 g  
Na<sub>2</sub>MoO<sub>4</sub> . 4H<sub>2</sub>O.....1.0 g  
Tap water.....1.0 L