

**ATCC medium: 897 Metal acetate medium**

Beijerinck's Solution (see below).....50.0 ml  
Phosphate Buffer (see below).....50.0 ml  
Trace Elements (see below).....1.0 ml  
Sodium acetate.....2.0 g  
Distilled water.....900.0 ml

Solid medium: add 15 g agar per liter. Liquid medium: omit agar; add phosphate aseptically after the medium has been autoclaved (to avoid precipitation). Autoclave at 121C for 15 min.

*Beijerinck's Solution:*

NH<sub>4</sub>Cl .....10.0 g  
MgSO<sub>4</sub> . 7H<sub>2</sub>O .....0.4 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....0.2 g  
Distilled water.....1.0 L

Dissolve CaCl<sub>2</sub> . 2H<sub>2</sub>O in 500 ml water and the other compounds in the second 500 ml water. Combine the two when all compounds have dissolved.

*Phosphate Buffer, pH 6.8:*

K<sub>2</sub>HPO<sub>4</sub> .....28.8 g  
KH<sub>2</sub>PO<sub>4</sub> .....14.4 g  
Distilled water.....1.0 L

*Trace Elements:*

EDTA.....50.0 g  
ZnSO<sub>4</sub> . 7H<sub>2</sub>O in 100 ml H<sub>2</sub>O.....22.0 g  
H<sub>3</sub>BO<sub>3</sub> in 200 ml H<sub>2</sub>O.....11.4 g  
MnCl<sub>2</sub> . 4H<sub>2</sub>O in 50 ml H<sub>2</sub>O.....5.06 g  
FeSO<sub>4</sub> . 7H<sub>2</sub>O in 50 ml H<sub>2</sub>O.....4.99 g  
CoCl<sub>2</sub> . 6H<sub>2</sub>O in 50 ml H<sub>2</sub>O.....1.61 g  
CuSO<sub>4</sub> . 5H<sub>2</sub>O in 50 ml H<sub>2</sub>O.....1.57 g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> . 4H<sub>2</sub>O in 50 ml H<sub>2</sub>O .....1.1 g  
Distilled water to.....1.0 L

Dissolve EDTA in 250 ml distilled water and heat to boiling until completely dissolved. Dissolve each of the remaining salts separately in volumes listed and mix together. Heat this solution to boiling, add the EDTA solution and continue to boil. Cool to 70C and adjust pH at this temperature with the addition of approximately 80-90 ml hot (70C) 20% KOH. Dilute solution to 1.0 L. Allow to stand in 2.0 L flask stoppered with cotton until solution turns purple (2 weeks). Filter out rust brown ppt. with Buchner funnel using 2 layers of Whatman #1 paper. Filter until clear. Refrigerate or freeze.

