

**ATCC medium: 1542 Benzoate nitrate salts medium (BNS)**

*Part A:*

Phosphate Buffer (see below).....200.0 ml  
NH<sub>4</sub>Cl.....0.3 g  
KNO<sub>3</sub>.....2.0 g  
Sodium benzoate.....1.0 g  
Distilled water.....500.0 ml

*Part B:*

MgSO<sub>4</sub> . 7H<sub>2</sub>O .....0.2 g  
CaCl<sub>2</sub> .....10.0 mg  
Trace Metals Solution (see below).....1.0 ml  
Distilled water.....299.0 ml

Dissolve and autoclave Parts A and B separately. Combine when cool.  
Adjust final pH to 8.2. Completely fill screw-capped tubes.

*Phosphate Buffer:*

K<sub>2</sub>HPO<sub>4</sub> .....5.12 g  
KH<sub>2</sub>PO<sub>4</sub> .....1.5 g

Dissolve in 200 ml distilled water. Adjust pH to 9.0 with KOH.

*Trace Metals Solution:*

Ferric EDTA (see below).....10.0 ml  
ZnSO<sub>4</sub> . 7H<sub>2</sub>O .....50.0 mg  
MnSO<sub>4</sub> . H<sub>2</sub>O .....50.0 mg  
CuSO<sub>4</sub> .....10.0 mg  
Cobalt nitrate.....10.0 mg  
Sodium borate.....10.0 mg  
Sodium molybdate.....200.0 ml  
Distilled water.....100.0 ml

*Ferric EDTA:*

EDTA.....17.9 g  
KOH.....3.23 g  
FeSO<sub>4</sub> . 7H<sub>2</sub>O .....13.7 g  
Distilled water.....550.0 ml

Dissolve EDTA and KOH in 186 ml distilled water. Dissolve FeSO<sub>4</sub> . 7H<sub>2</sub>O  
in 364 ml distilled water. Mix the two solutions and bubble with air  
overnight to oxidize the Fe<sub>2+</sub> to Fe<sub>3+</sub>. Store in a dark place.