

**ATCC medium: 2159 BT medium**

KH <sub>2</sub> PO <sub>4</sub> .....	0.2 g
NH <sub>4</sub> Cl .....	0.3 g
KCl.....	0.5 g
NaCl.....	1.0 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.15 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.4 g
Resazurin.....	0.5 mg
SL-10 Trace Elements Solution (see below).....	1.0 ml
3-Hydroxybenzoate Solution (see below).....	10.0 ml
Yeast Extract Solution (see below).....	5.0 ml
Trypticase Solution (see below).....	5.0 ml
NaHCO <sub>3</sub> Solution (see below) .....	50.0 ml
Selenite/Tungstate Solution (see below).....	1.0 ml
Na <sub>2</sub> S Solution (see below) .....	13.0 ml
Distilled water.....	1.0 L

Do not add the solutions to the initial basal medium. Autoclave the basal medium at 121C for 15 minutes. Cool under 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Aseptically add the solutions listed. Adjust final pH to 7.6 with NaCO<sub>3</sub>.

**SL-10 Trace Elements Solution:**

HCl (25%).....	10.0 ml
FeCl <sub>2</sub> . 4H <sub>2</sub> O .....	1.5 g
ZnCl <sub>2</sub> .....	70.0 mg
MnCl <sub>2</sub> . 4H <sub>2</sub> O .....	100.0 mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0 mg
CoCl <sub>2</sub> . 6H <sub>2</sub> O .....	190.0 mg
CuCl <sub>2</sub> . 2H <sub>2</sub> O .....	2.0 mg
NiCl <sub>2</sub> . 6H <sub>2</sub> O .....	24.0 mg
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	36.0 mg
Distilled water.....	990.0 ml

Dissolve FeCl<sub>2</sub> in the HCl, dilute with water, add and dissolve the other salts; adjust pH to 6.0 with NaOH, and fill to 1.0 L with distilled water.

**3-Hydroxybenzoate Solution:**

3-Hydroxybenzoate (free acid).....	2.8 g
Distilled water.....	10.0 ml

Neutralize with NaOH. Filter-sterilize.

*Yeast Extract Solution:*

Yeast extract.....10.0 g  
Distilled water.....100.0 ml

Filter-sterilize.

*Trypticase Solution:*

Trypticase.....10.0 g  
Distilled water.....100.0 ml

Filter-sterilize.

*NaHCO<sub>3</sub> Solution:*

NaHCO<sub>3</sub>.....10.0 g  
Distilled water.....100.0 ml

Equilibrate with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas. Filter-sterilize.

*Selenite/Tungstate Solution:*

NaOH.....0.05 g  
NaSeO<sub>3</sub>.....30.0 mcg  
Na<sub>2</sub>WO<sub>4</sub>.....40.0 mcg  
Distilled water.....100.0 ml

Filter-sterilize or autoclave at 121C for 15 minutes.

*Na<sub>2</sub>S Solution:*

Na<sub>2</sub>S . 9H<sub>2</sub>O .....0.6 g  
Distilled water.....20.0 ml

Filter-sterilize.