

**ATCC medium: 2657 PMSY Medium**

Na <sub>2</sub> HPO <sub>4</sub> (0.5M).....	25.00	ml
NaHPO <sub>4</sub> (0.5M).....	25.00	ml
(NH <sub>4</sub> )SO <sub>4</sub> (10%).....	10.00	ml
Concentrated Base (See Below).....	1.00	ml
Na-Thiosulfate (0.1M).....	1.00	ml
Yeast extract.....	3.00	g
Distilled water.....	928.00	ml

Autoclave at 121°C for 15 minutes. After cooling add 10.0 ml of a Filter sterilized stock solution of 1M Na-Succinate (2.7g/10ml).

**Concentrated base: per liter**

Nitriltriacetic acid (NTA -free acid),.....	20.0	g
MgSO <sub>4</sub> anhydrous,.....	28.9	g
CaCl <sub>2</sub> ·2H <sub>2</sub> O,.....	6.7	g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O.....	18.5	mg
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	198.0	g
Hutner's Metal; 44 (see below).....	100.0	ml

Dissolve NTA separately in 600 ml water and neutralize with KOH (14.6 g KOH) add other components and dissolve in order given. Adjust to pH 6.8 before making to a final volume of 1 liter. A precipitate forms when adjusting the pH from the acid side of 6.8 with KOH (need about 100 ml of 1M KOH ), but eventually will redissolve with stirring. When the pH is near 6.8, the color of the solution changes from a deep yellow to straw color. The final base may be kept for at least a year. Store at 4 °C with a layer of toluene on top to prevent contamination.

**Hutner's Metal 44:**

Dissolve the following in order. Do not add components until the previous one has completely dissolved.

Distilled water.....	800.00	ml
EDTA (free acid, not sodium salt, warm to dissolve).....	2.50	g
ZnSO <sub>4</sub> 7H <sub>2</sub> O.....	10.95	g
FeSO <sub>4</sub> . 7 H <sub>2</sub> O.....	5.00	g
MnSO <sub>4</sub> . H <sub>2</sub> O.....	1.54	g
CuSO <sub>4</sub> . 5 H <sub>2</sub> O.....	0.39	g
Co(NO <sub>3</sub> ) <sub>2</sub> . 6 H <sub>2</sub> O.....	0.25	g

$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$ ..... 0.18 g

*Add a few drops of conc.  $\text{H}_2\text{SO}_4$  to retard precipitation. Make to a final volume of 1 liter, it should be a clear, lime green solution. Protect from light by wrapping the bottle with Aluminum Foil. Store indefinitely*