

### **ATCC Medium: 2479 4YACO Medium**

NaCl.....	1.0 g
KCl.....	0.5 g
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.4 g
CaCl <sub>2</sub> ·2H <sub>2</sub> O.....	0.1 g
NH <sub>4</sub> Cl.....	0.3 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.2 g
NaSO <sub>4</sub> .....	0.15 g
0.1 M MOPS* (pH 7.2).....	100.0 ml
Resazurin.....	0.1 mg
DI Water.....	848 ml

\*3-N-morpholino propanesulfonate

Adjust pH to 7.2-7.4 then autoclave at 121°C. Cool under 80% N<sub>2</sub>-20% CO<sub>2</sub>.

Prior to inoculation, the medium is reduced by adding 100.0 mM dithiothreitol (separately sterilized under 100% N<sub>2</sub> to a final concentration of 1.0 mM). The headspace is exchanged with 80% H<sub>2</sub>-20% CO<sub>2</sub> for dispensing into culture tubes. The headspace should be at least four times the liquid volume.

Filter-sterilize and aseptically add the following solutions:

1.0 M NaHCO <sub>3</sub> ( <i>gas under CO<sub>2</sub></i> ).....	60.0 ml
Trace Element Mixture w/EDTA ( <i>see below</i> ).....	1.0 ml
Six Vitamin Solution ( <i>see below</i> ).....	1.0 ml
Vitamin B <sub>12</sub> ( <i>see below</i> ).....	1.0 ml
Co-Factor Mixture ( <i>see below</i> ).....	20.0 ml
Yeast Autolysate ( <i>see below</i> ).....	40.0 ml

#### **Trace Element Mixture w/ EDTA**

Na EDTA.....	5.2 g
FeSO <sub>4</sub> ·7H <sub>2</sub> O.....	2100.0 mg
H <sub>3</sub> BO <sub>3</sub> .....	30.0 mg
MnCl <sub>2</sub> ·4H <sub>2</sub> O.....	100.0 mg
CoCl <sub>2</sub> ·6H <sub>2</sub> O.....	190.0 mg
NiCl <sub>2</sub> ·6H <sub>2</sub> O.....	24.0 mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	2.0 mg
ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	144.0 mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	1200.0 mg
DI Water.....	999.0 ml

Adjust pH to 6.0 with NaOH. Deoxygenate; sterilize under 100% N<sub>2</sub>.

### **Six-Vitamin Solution**

4-Aminobenzoic acid.....	4.0 mg
Nicotinic acid.....	10.0 mg
Calcium-D-(+)-pantothenate.....	5.0 mg
Pyridoxine HCl.....	15.0 mg
Thiamine HCl.....	10.0 mg
Biotin.....	1.0 mg
DI Water.....	100.0 ml

While being stirred, the mixture is neutralized drop wise by addition of 0.1 M NaOH which facilitates solubility of all vitamins. Deoxygenate; filter-sterilize under N<sub>2</sub>.

### **Vitamin B<sub>12</sub>**

Vitamin B <sub>12</sub> (cyanocobalamin).....	5.0 mg
DI Water.....	100.0 ml

Deoxygenate; filter-sterilize and store under N<sub>2</sub>. Protect from light.

### **Co-Factor Mixture**

Pyridoxal HCl.....	25.0 mg
Pyridoxal phosphate.....	5.0 mg
Ca folinic acid.....	25.0 mg
beta-NAD.....	5.0 mg
Coenzyme A.....	5.0 mg
FAD.....	5.0 mg
Nicotinamide.....	2.5 mg
Folic acid.....	250.0 ug
Riboflavin.....	50.0 ug
Thiamine pyrophosphate.....	250.0 mg
DI Water.....	100.0 ml

Deoxygenate; filter-sterilize and store under N<sub>2</sub>.

### **Yeast Autolysate**

Allow 56.0 g of dry baker's yeast( Red Star Yeast only) to autolyze in 200.0 ml distilled water for 24 hrs at 56°C. Remove cell debris by centrifugation. Neutralize the supernatant liquid with 5.0 N NaOH while stirring. Deoxygenate with N<sub>2</sub>; filter-sterilize and store under N<sub>2</sub>.