

### **ATCC Medium: 1869 PRAS-PYG w/Tween 80**

Peptone.....	5.0 g
Trypticase.....	5.0 g
Yeast extract.....	10.0 g
0.025% Resazurin.....	4.0 ml
Salt solution (see below).....	40.0 ml
Hemin solution (see below).....	10.0 ml
Vitamin K <sub>1</sub> solution (see below).....	0.2 ml
Glucose.....	10.0 g
Tween 80.....	0.25 ml
L-Cysteine HCl x H <sub>2</sub> O.....	0.5 g
DI Water.....	945.55 ml

Boil medium without glucose, tween 80, and cysteine. Cool under 97% N<sub>2</sub>, 3% H<sub>2</sub> and add glucose, tween 80, and cysteine. Adjust pH to 7.0. Dispense anaerobically under same gas phase. Autoclave at 121°C.

#### **Salts Solution**

CaCl <sub>2</sub> .....	0.2 g
MgSO <sub>4</sub> .....	0.2 g
K <sub>2</sub> HPO <sub>4</sub> .....	1.0 g
KH <sub>2</sub> PO <sub>4</sub> .....	1.0 g
NaHCO <sub>3</sub> .....	10.0 g
NaCl.....	2.0 g
DI Water.....	1000 ml

Mix calcium chloride and magnesium sulfate in 300 ml of distilled water until dissolved. Add 500 ml of distilled water and add the remaining salts while swirling slowly. Add 200 ml of distilled water, mix, and store at 4°C.

#### **Hemin Solution**

Hemin.....	50.0 mg
1N NaOH.....	1.0 ml
DI Water.....	99 ml

Dissolve hemin in NaOH and raise volume to 100ml with distilled water.

#### **Vitamin K<sub>1</sub> Solution**

Vitamin K <sub>1</sub> .....	0.15 ml
95% Ethanol.....	30.0 ml

Dissolve Vitamin K<sub>1</sub> in ethanol. Store in brown bottle and keep refrigerated. Discard after one month.