

## **ATCC Medium: 2868 Modified TY Anaerobic Complex Medium**

### **Solution A**

Trypticase.....	30 g
Yeast Extract.....	20 g
Haemin.....	0.005 g
Resazurin (0.025%).....	4 ml
Reducing Agent (add aseptically; see below).....	200 ml
DI Water.....	800 ml

Combine the above ingredients and bring to a boil in a round-bottom flask while flushing with O<sub>2</sub>-free gas. Seal flask closed, and autoclave at 121°C. After autoclaving, allow to cool, open without admitting oxygen and while flushing with gas, add the sterile Reducing Agent. The medium color becomes colorless in 10-30 min, indicating a drop in Eh. Dispense into appropriate vessel, close with sterile rubber septum and seal with aluminum cap.

Note: Solution A and Reducing Agent are prepared separately to avoid precipitation and chemical interactions during autoclaving.

### **Reducing Agent (x100)**

NH <sub>4</sub> Cl.....	0.4 g
MgCl <sub>2</sub> x 6H <sub>2</sub> O.....	0.1 g
CaCl <sub>2</sub> x 2H <sub>2</sub> O.....	0.05 g
L-Cysteine HCl.....	0.5 g
FeCl <sub>2</sub> x 4H <sub>2</sub> O.....	0.05 g
DI Water.....	200 ml

Dissolve L-Cysteine HCl, then FeCl<sub>2</sub> x 4H<sub>2</sub>O and mix with other components in a serum bottle. Flash with nitrogen immediately. Autoclave at 121°C.

### **Anaerobic cultures preservation**

The most common storage procedure is to leave liquid culture at 4°C, where it may be viable for several months (Monaghan et. al., 1999). For longer periods, anaerobic stocks mixed with glycerol can be stored between -70°C and -80°C (Bryukhanov, Netrusov, 2006).