

### **ATCC Medium: 2850 Modified Nitrate Minimal Salts Medium**

NaCl.....	20.0 g
MgSO <sub>4</sub> x 7H <sub>2</sub> O.....	1.0 g
CaCl <sub>2</sub> x 6H <sub>2</sub> O.....	0.2 g
KNO <sub>3</sub> .....	1.0 g
Chelated Iron Solution.....	2.0 ml
Trace Elements Solution (see below).....	10 ml
Vitamin Solution (see below).....	10 ml
KH <sub>2</sub> PO <sub>4</sub> .....	0.272 g
Na <sub>2</sub> HPO <sub>4</sub> x 12H <sub>2</sub> O.....	0.717 g
Purified Agar (eg. Oxoid L28).....	12.5 g
DI Water.....	980.0 ml

Adjust pH to 6.8 and autoclave at 121 C.

#### **Trace Elements Solution**

Nitrilotriacetic acid.....	1.50 g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O.....	3.00 g
MnSO <sub>4</sub> x H <sub>2</sub> O.....	0.50 g
NaCl.....	1.00 g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O.....	0.10 g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O.....	0.18 g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O.....	0.10 g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O.....	0.18 g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O.....	0.01 g
KAl(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O.....	0.02 g
H <sub>3</sub> BO <sub>3</sub> .....	0.01 g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O.....	0.01 g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O.....	0.03 g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O.....	0.30 mg
DI Water.....	1000.00 ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

#### **Vitamin solution**

Biotin.....	2.00 mg
Folic acid.....	2.00 mg
Pyridoxine-HCl.....	10.00 mg
Thiamine-HCl x 2 H <sub>2</sub> O.....	5.00 mg
Riboflavin.....	5.00 mg
Nicotinic acid.....	5.00 mg
D-Ca-pantothenate.....	5.00 mg
Vitamin B12.....	0.10 mg

p-Aminobenzoic acid.....5.00 mg  
Lipoic acid.....5.00 mg  
DI Water.....1000.00 ml

**Chelated Iron Solution**

Ferric(III)ammonium citrate\* .....0.1 g  
EDTA, sodium salt.....0.2 g  
DI Water.....100 ml  
HCl(conc).....0.3 ml

Use 2ml of this chelated iron solution per liter of final medium.

\*0.05g of Ferric(III)chloride may be substituted.