

## **ATCC Medium: 2823 Modified Methylophaga Agar/Broth Medium**

### **Complete Medium**

Mineral Base (see below).....	100.0 ml
Solution T (see below).....	2.0 ml
Vitamin B12 (0.1 mcg/ml) filter-sterilized.....	1.0 ml
Methanol (filter-sterilized).....	10.0 ml

Aseptically combine each solution.

For solid medium prepare a 2X Mineral Base solution and a 3% agar solution and autoclave both separately. Cool to 50C and aseptically mix the solutions in equal volume before adding solution T, the B12 solution and methanol.

### **Mineral Base**

NaCl.....	24.0 g
MgCl <sub>2</sub> x 6H <sub>2</sub> O.....	3.0 g
MgSO <sub>4</sub> x 7H <sub>2</sub> O.....	2.0 g
KCl.....	0.5 g
CaCl <sub>2</sub> x 2H <sub>2</sub> O.....	1.0 g
Bis-Tris.....	0.5 g
Wolfe's Mineral Solution (see below).....	10.0 ml
DI Water.....	990.0 ml

Adjust to pH 7.3 with HCl. Autoclave at 121C.

### **Solution T**

KH <sub>2</sub> PO <sub>4</sub> .....	0.7 g
NH <sub>4</sub> Cl.....	10.0 g
Bis-Tris.....	10.0 g
Ferric ammonium citrate.....	0.3 g
DI Water.....	100.0 ml

Adjust to pH 7.3. Autoclave at 121C.

### **Wolfe's Mineral Solution**

Nitriloacetic Acid.....	1.5 g
MgSO <sub>4</sub> .....	3.0 g
MnSO <sub>4</sub> .....	0.5 g
NaCl.....	1.0 g
FeSO <sub>4</sub> .....	0.1 g
CaCl <sub>2</sub> .....	0.1 g
CoCl <sub>2</sub> .....	0.1 g
ZnSO <sub>4</sub> .....	0.1 g
CuSO <sub>4</sub> .....	0.01 g

$\text{AlK}(\text{SO}_4)_2$ .....	0.01 g
$\text{H}_3\text{BO}_4$ .....	0.01 g
$\text{Na}_2\text{MoO}_4$ .....	0.01 g
DI Water.....	1000 ml