

## **ATCC Medium: 2846 F/2 Medium**

### **Complete Medium**

NaNO <sub>3</sub> Solution (see below).....	1 ml
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O Solution (see below).....	1 ml
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O Solution (see below).....	1 ml
Trace Metals Solution (see below).....	1 ml
Vitamin B12 Solution (see below; add aseptically).....	1 ml
Biotin Vitamin Solution (see below; add aseptically).....	1 ml
Thiamin Vitamin Solution (see below; add aseptically)..	1 ml
DI Water.....	993 ml

To approximately 993 mL of non-pasteurized seawater (30-35ppt), add each of the components in the order specified (except vitamins) while stirring continuously. \*For 1.5% agar medium add 15 g of agar into the flask; do not mix. Cover and autoclave medium at 121C. When cooled add sterile vitamins. Store at refrigerator temperature.

### **NaNO<sub>3</sub> Solution**

NaNO <sub>3</sub> .....	7.5 g
DI Water.....	100 ml

### **NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O Solution**

NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O.....	0.5 g
DI Water.....	100 ml

### **Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O Solution**

Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O.....	3.0 g
DI Water.....	100 ml

### **Trace Metals Solution**

ZnSO <sub>4</sub> ·7H <sub>2</sub> O.....	23 mg
MnSO <sub>4</sub> ·H <sub>2</sub> O.....	152 mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O.....	7.3 mg
CoSO <sub>4</sub> ·7H <sub>2</sub> O.....	14 mg
CuCl <sub>2</sub> ·2H <sub>2</sub> O.....	6.8 mg
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O.....	4.6 g
Na <sub>2</sub> EDTA·2H <sub>2</sub> O.....	4.4 g
DI Water.....	500 ml

Begin stirring and heating 450 ml DI Water. When the stirring water approaches the boiling point, add the following components in the order listed. Bring the volume to 500 ml with DI Water. Store at refrigerator temperature.

**Vitamin B12 Solution**

HEPES buffer pH 7.8 (Sigma H-3375).....2.4 g  
Vitamin B<sub>12</sub> (cyanocobalamin, Sigma V-6629).....0.027 g  
DI Water.....200 ml

Prepare 200 mL of HEPES buffer (50 mM). Adjust the pH to 7.8. Add Vitamin B<sub>12</sub> (0.1 mM) wait until fully dissolved. Sterilize by 0.45 µm Millipore filter. Store in dark at freezer temperature.

\*\* The amount of vitamins added can vary from medium to medium so the final concentration is not listed.

**Biotin Vitamin Solution**

HEPES buffer pH 7.8 (Sigma H-3375).....2.4 g  
Biotin.....0.005 g  
DI Water.....200 ml

Prepare 200 mL of HEPES buffer (50 mM). Adjust the pH to 7.8. Add biotin (0.1 mM) wait until fully dissolved. Sterilize by 0.45 µm Millipore filter. Store in dark at freezer temperature.

\*\* The amount of vitamins added can vary from medium to medium so the final concentration is not listed.

**Thiamin Vitamin Solution**

HEPES buffer pH 7.8 (Sigma H-3375).....1.2 g  
Thiamine.....0.11 g  
DI Water.....100 ml

Prepare 100 mL of HEPES buffer (50 mM). Adjust the pH to 7.8. Add Thiamine (1 mM) wait until fully dissolved. Sterilize by 0.45 µm Millipore filter. Store in dark at freezer temperature.

\*\* The amount of vitamins added can vary from medium to medium so the final concentration is not listed.