

# LI MEDIUM FOR CULTURING PYROCYSTIS

## 1. Stock solutions for major elements

$\text{NaNO}_3$	7.5 g/100 mL
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.5 g/100 mL
$\text{Na}_2\text{SiO}_3 \cdot 9 \text{H}_2\text{O}^*$	3.0 g/100 mL

\*  $\text{Na}_2\text{SiO}_3$  = di-Sodium-**metasilicate**.

## 2. Primary stock solutions for trace elements

$\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$	18.0 g/100 mL
$\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$	2.2 g/100 mL
$\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$	1.0 g/100 mL
$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	0.245 g/100 mL
$\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$	1.99 g/100 mL
$\text{H}_2\text{SeO}_3$	0.13 g/100 mL
$\text{NiSO}_4 \cdot 6 \text{H}_2\text{O}$	0.27 g/100 mL
$\text{Na}_3\text{VO}_4$	0.184 g/100 mL
$\text{K}_2\text{CrO}_4$	0.194 g/100 mL

## 3. Trace metal working stock solution

- 1) Dissolve 4.36 g  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  and 3.15 g  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$  in ca. 900 mL  $\text{H}_2\text{O}$  in a 1000-mL volumetric flask.
- 2) Add 1 mL of each trace metal primary stock solution.
- 3) Bring to 1000 mL with  $\text{H}_2\text{O}$ .
- 4) Autoclave

#### **4. Vitamin stock solution**

Biotin 0.01 g/100 mL

Cyanocobalamine (B<sub>12</sub>) 0.1 g/100 mL

Note: Vitamin B<sub>12</sub> and Biotin are obtained in a crystalline form. When preparing the Vitamin B<sub>12</sub> Stock Solution allow for approximately 11% water of crystallization (For each 1.0 mg of Vitamin B<sub>12</sub> add 0.89 ml dH<sub>2</sub>O). When preparing the Biotin Stock Solution allow for approximately 4% water of crystallization (For each 1.0 mg of Biotin add 9.6 ml dH<sub>2</sub>O).

Keep the vitamin solutions frozen. Bottles of polyethylene are recommended for storage of vitamins.

#### **5. Vitamin working stock solution**

- 1) Dissolve 20 mg Thiamine HCl (Vitamin B<sub>1</sub>) in ca. 80 mL dH<sub>2</sub>O in a 100 mL volumetric flask.
- 2) Add 1 mL of the biotin primary stock solution.
- 3) Add 0.1 mL of the cyanocobalamin primary stock solution.
- 4) Fill with dH<sub>2</sub>O to 100 mL.

The vitamin working stock solution is divided into to 10-mL lots in polyethylene vials and kept frozen until use.

#### **6. Final preparation of L-medium**

Add to 1 liter seawater:

1.0 mL NaNO<sub>3</sub> stock solution.

1.0 mL NaH<sub>2</sub>PO<sub>4</sub>•H<sub>2</sub>O stock solution.

1.0 mL Na<sub>2</sub>SiO<sub>3</sub>•9 H<sub>2</sub>O stock solution. (For diatoms and silicoflagellates only. Otherwise leave out).

1.0 mL Trace metal working stock solution.

0.5 mL Vitamin working stock solution.

Autoclave medium