

# ESAW Media for Marine Phytoplankton Version 3

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## Abstract

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## Before start

Be sure to mix and autoclave **solution I** and **solution II** separately before combining and adding trace metal solutions and vitamin solutions. Store media in 4°C. Note, Vitamin stock solutions amounts are per mL Trace Metal stock solution amounts are per one liter. Adjust amounts, as needed, according to desired stock amount.

## Protocol

### SOLUTION I

#### Step 1.

Na<sub>2</sub>SO<sub>4</sub>

 **AMOUNT**

3 g Additional info:

 **NOTES**

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Make Solutions I and II separately.

### SOLUTION I

#### Step 2.

NaCl

 **AMOUNT**

21 g Additional info:

### SOLUTION I

#### Step 3.

KCL

 **AMOUNT**

1 g Additional info:

#### SOLUTION I

##### Step 4.

$\text{NaHCO}_3$

 **AMOUNT**

0 g Additional info:

#### SOLUTION I

##### Step 5.

KBr

 **AMOUNT**

0 g Additional info:

#### SOLUTION I

##### Step 6.

$\text{H}_3\text{BO}$

 **AMOUNT**

0 g Additional info:

#### SOLUTION I

##### Step 7.

NaF (2.7 g/L stock)

 **AMOUNT**

1 ml Additional info:

#### SOLUTION I

##### Step 8.

Bring to 500 mL MilliQ water

#### SOLUTION II

##### Step 9.

$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$

 **AMOUNT**

9 g Additional info:

 **NOTES**

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Make Solutions I and II separately.

#### SOLUTION II

##### Step 10.

CaCl<sub>2</sub>-2H<sub>2</sub>O

 [AMOUNT](#)

1 g Additional info:

SOLUTION II

**Step 11.**

SrCl<sub>2</sub>-6H<sub>2</sub>O

 [AMOUNT](#)

0 g Additional info:

SOLUTION II

**Step 12.**

Tris-HCL (pH 7.8) (1.0 M stock)

 [AMOUNT](#)

5 ml Additional info:

SOLUTION II

**Step 13.**

Fe-EDTA

 [AMOUNT](#)

1 ml Additional info:

 [PROTOCOL](#)

. [Fe-EDTA Stock solution for ESAW Media for Marine Phytoplankton](#)

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Fe-EDTA Stock solution, amounts per one Liter (1000 mL)

**Step 13.1.**

Na<sub>2</sub>EDTA

 [AMOUNT](#)

4 g Additional info:

Fe-EDTA Stock solution, amounts per one Liter (1000 mL)

**Step 13.2.**

FeCl<sub>3</sub>

 [AMOUNT](#)

3 g Additional info:

Fe-EDTA Stock solution, amounts per one Liter (1000 mL)

**Step 13.3.**

Bring solution to 1000 mL with MilliQ H<sub>2</sub>O and filter sterilize with a 0.2µm filter. Store in the dark at 4°C

## SOLUTION II

### Step 14.

$K_2HPO_4$  (10g/L stock)

 [AMOUNT](#)

1 ml Additional info:

## SOLUTION II

### Step 15.

$NaNO_3$  (550 mM stock)

 [AMOUNT](#)

1 ml Additional info:

## SOLUTION II

### Step 16.

Selenite (10  $\mu$ M stock)

 [AMOUNT](#)

1 ml Additional info:

## SOLUTION II

### Step 17.

Bring to 500 mL MilliQ water

## Autoclave

### Step 18.

Autoclave **Solution I** and **Solution II** separately

## After Cooling

### Step 19.

Combine **Solution I** and **Solution II**

## Trace Metals

### Step 20.

Add trace Metals Solutions

 [AMOUNT](#)

1 ml Additional info:

 [PROTOCOL](#)

. [Trace Metal Solution for ESAW Media](#)

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 [NOTES](#)

See protocol, amounts are per one liter.

TM Solution, amounts per one Liter (1000 mL)

**Step 20.1.**



 AMOUNT

10 mg Additional info:

TM Solution, amounts per one Liter (1000 mL)

**Step 20.2.**



 AMOUNT

22 mg Additional info:

TM Solution, amounts per one Liter (1000 mL)

**Step 20.3.**

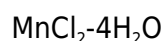


 AMOUNT

10 mg Additional info:

TM Solution, amounts per one Liter (1000 mL)

**Step 20.4.**



 AMOUNT

180 mg Additional info:

TM Solution, amounts per one Liter (1000 mL)

**Step 20.5.**



 AMOUNT

6 mg Additional info:

TM Solution

**Step 20.6.**

After mixing, filter sterilize with 0.2  $\mu\text{m}$  filter, and store in dark at 4°C

Vitamin

**Step 21.**

Add Vitamin Solution

 AMOUNT

0 ml Additional info:

## [PROTOCOL](#)

### [. Vitamin Solution for ESAW Media for Marine Phytoplankton](#)

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## [NOTES](#)

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See protocol, amounts are per one mL

Vitamin solution, amounts per one mL

#### **Step 21.1.**

Thiamine

## [AMOUNT](#)

100 mg Additional info:

Vitamin solution, amounts per one mL

#### **Step 21.2.**

Vitamin B<sub>12</sub>

## [AMOUNT](#)

2 mg Additional info:

Vitamin solution, amounts per one mL

#### **Step 21.3.**

Biotin

## [AMOUNT](#)

1 mg Additional info:

Vitamin solution

#### **Step 21.4.**

After mixing, filter sterilize with 0.2 µm filter and store in dark at -20°C. Divide into small aliquots if desired.