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# Provasoli Enriched Seawater (PES) medium solution

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol outlines the steps required to prepare Provasoli Enriched Seawater (PES) medium in accordance with the method by Provasoli (1966).

CITATION

L. Provasoli (1966). Media and prospects for the cultivation of marine algae. Proceedings of the US-Japan Conference.
LINK
https://cir.nii.ac.jp/crid/1572824499547489536

## Protocol materials

- Biotin **P212121** Step 4.3
- Sodium glycerophosphate hydrate **Merck MilliporeSigma (Sigma-Aldrich)** Step 3.5
- Cobalt(II) sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich)** Step 1.5
- HEPES Sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**H7006 Step 3.1
- Manganese sulfate, monohydrate **Bio Basic Inc. Catalog #**MB0334.SIZE.500g Step 1.3
- Zinc sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**204986 Step 1.4
- MilliQ water In 6 steps
- Boric acid **Fisher Scientific Catalog #**BP1681 Step 1.2
- Ammonium iron(II) sulfate hexahydrate **Bio Basic Inc. Catalog #**AB0065.SIZE.500g Step 2.2
- Sodium nitrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S5506 Step 3.4
- Titriplex® III solution **Merck MilliporeSigma (Sigma-Aldrich)** In 2 steps
- Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**44944 Step 1.6
- Potassium iodide Step 3.6
- Vitamin B12 **Fisher Scientific Catalog #**68-19-9 Step 4.1
- Thiamine HCl **P212121** Step 4.2

## Before start

**It is important to follow the order of chemicals added as shown in the protocol.**

## P II solution

1 5000 mL MilliQ water **Sigma Aldrich**

1.1 Add 5 g Titriplex® III solution **Sigma Aldrich**

1.2 Add 5.7 g Boric acid **Sigma Aldrich** **Catalog #**BP1681

1.3 Add 0.62 g
Manganese sulfate, monohydrate **Sigma Aldrich** **Catalog #**MB0334.SIZE.500g

1.4 Add 0.11 g Zinc sulfate heptahydrate **Sigma Aldrich** **Catalog #**204986

1.5 Add 0.024 g Cobalt(II) sulfate heptahydrate **Sigma Aldrich**

1.6 Add 0.245 g Iron(III) chloride hexahydrate **Sigma Aldrich** **Catalog #**44944

## Fe-solution

2 5000 mL MilliQ water **Sigma Aldrich**

2.1 Add 3.3 g Titriplex® III solution **Sigma Aldrich**

2.2 Add 3.51 g
Ammonium iron(II) sulfate hexahydrate **Sigma Aldrich** **Catalog #**AB0065.SIZE.500g

Note

Solution equals 0.1 mg $Fe^{2+}$ x $ml^{-1}$

## Primary solution

2h

3 6000 mL MilliQ water **Sigma Aldrich**

3.1 Add 23.4 g HEPES Sodium salt **Sigma Aldrich Catalog #**H7006 (or 30 g Tris buffer)

3.2 Add 1500 mL P II-solution go to step #1

3.3 Add 1500 mL Fe-solution go to step #2

3.4 Add 21 g Sodium nitrate **Sigma Aldrich Catalog #**S5506

3.5 Add 2.12 g
Sodium glycerophosphate hydrate **Merck MilliporeSigma (Sigma-Aldrich)**

3.6 Add 16 mg Potassium iodide **Sigma Aldrich**

3.7 Pasteurise for 02:00:00 at 99 °C

2h

## Vitamins

4 Vitamins to be added through a sterile single injection with a front filter (0.2 µm) in the cooled off primary solution (maximum temperature of 60 °C ).

4.1 30 mL Vitamin B12 **Sigma Aldrich** **Catalog #**68-19-9 (10 mg per 500 ml MilliQ water **Sigma Aldrich** )

4.2 30 mL Thiamine HCl **Sigma Aldrich** (500 mg per 500 ml MilliQ water **Sigma Aldrich** )

4.3 30 mL Biotin **Sigma Aldrich** (5 mg per 500 ml MilliQ water **Sigma Aldrich** )

## Storage

5 Store PES in Duran bottles in a cold and dark setting at 10 °C .

| Equipment | |
|---|---|
| **Duran® laboratory bottles, with caps** | NAME |
| Glass bottle | TYPE |
| Duran | BRAND |
| Z305197-10EA | SKU |
| 500 ml | SPECIFICATIONS |

## Application

6 Culturing medium is created by adding 200 mL PES to 10 L sterile seawater.

## Citations

L. Provasoli. Media and prospects for the cultivation of marine algae
**https://cir.nii.ac.jp/crid/1572824499547489536**