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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 (Invalid date). Media and prospects for the cultivation of marine algae.
Proceedings of the US-Japan Conference.

<https://cir.nii.ac.jp/crid/1572824499547489536>

[LINK](#)



Protocol materials

⊗ MilliQ water

⊗ MilliQ water

⊗ Titriplex® III solution **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ Boric acid **Fisher Scientific Catalog #BP1681**

⊗ Manganese sulfate, monohydrate **Bio Basic Inc. Catalog #MB0334.SIZE.500g**

⊗ Zinc sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #204986**

⊗ Cobalt(II) sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944**

⊗ Titriplex® III solution **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ Ammonium iron(II) sulfate hexahydrate **Bio Basic Inc. Catalog #AB0065.SIZE.500g**

⊗ HEPES Sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H7006**

⊗ Vitamin B12 **Fisher Scientific Catalog #68-19-9**

⊗ MilliQ water

⊗ Thiamine HCl **P212121**

⊗ MilliQ water

⊗ Biotin **P212121**

⊗ MilliQ water

⊗ MilliQ water

⊗ Sodium nitrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5506**

⊗ Sodium glycerophosphate hydrate **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ Potassium iodide

Troubleshooting

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

1.1

Add



5 g

Titriplex® III solution **Merck MilliporeSigma (Sigma-Aldrich)**

1.2

Add



5.7 g

Boric acid **Fisher Scientific Catalog #BP1681**

1.3

Add



0.62 g

Manganese sulfate, monohydrate **Bio Basic Inc. Catalog #MB0334.SIZE.500g**

1.4

Add



0.11 g

Zinc sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #204986**

1.5

Add



0.024 g

Cobalt(II) sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich)**

1.6

Add



0.245 g

Iron(III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944**

Fe-solution

2



5000 mL



MilliQ water

2.1

Add



3.3 g

Titriplex® III solution **Merck MilliporeSigma (Sigma-Aldrich)**



2.2 Add  3.51 g

 Ammonium iron(II) sulfate hexahydrate **Bio Basic Inc. Catalog #AB0065.SIZE.500g**

Note

Solution equals  0.1 mg $\text{Fe}^{2+} \times \text{ml}^{-1}$

Primary solution

2h

3  6000 mL  MilliQ water

3.1 Add  23.4 g

 HEPES Sodium salt **Merck MilliporeSigma (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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5506**

3.5 Add  2.12 g

 Sodium glycerophosphate hydrate **Merck MilliporeSigma (Sigma-Aldrich)**

3.6 Add  16 mg  Potassium iodide

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 Fisher Scientific Catalog #68-19-9 (10 mg per 500 ml MilliQ water)

4.2 30 mL Thiamine HCl P212121 (500 mg per 500 ml MilliQ water)

4.3 30 mL Biotin P212121 (5 mg per 500 ml MilliQ water)

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>