



Version 1

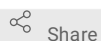
Jul 12, 2021

Preparation of defined medium for marine holozoans.

V.1

Sebastian R R Najle¹, Eduard Ocaña-Pallarès², Iñaki Ruiz-Trillo²¹Center for Genomic Regulation (CRG); ²Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra)

1 Works for me



Share

dx.doi.org/10.17504/protocols.io.wqgfdtw

Protist Research to Optimize Tools in Genetics (PROT-G) Multicellgenomelab



Sebastian R Najle

Center for Genomic Regulation (CRG)

ABSTRACT

In order to study specific metabolic pathways in microorganisms is crucial to have complete control on the components of the growth medium. This protocol describes the components and mode of preparation of a minimum medium (low nutrients medium) of defined composition, suited for marine holozoans (named "modified L1 medium" - mL1). mL1 medium can be used for cultivation of different marine holozoans (i.e. Ichthyosporea and Corallochytraea), allowing to completely control the components (for example: carbon source, nitrogen source, etc). mL1 medium is based on the L1 medium recipe publically available at the National Center for Marine Algae and Microbiota (<http://ncma.bigelow.org/algal-recipes>).

DOI

dx.doi.org/10.17504/protocols.io.wqgfdtw

PROTOCOL CITATION

Sebastian R R Najle, Eduard Ocaña-Pallarès, Iñaki Ruiz-Trillo 2021. Preparation of defined medium for marine holozoans.. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.wqgfdtw>

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jan 02, 2019

LAST MODIFIED

Jul 12, 2021

PROTOCOL INTEGER ID

18920

MATERIALS TEXT

MATERIALS

[Biotin P212121](#)

[Thiamine HCl P212121](#)

[Potassium chromate Contributed by users](#)

[Selenious acid P212121](#)

[Ethylenediaminetetraacetic acid disodium salt dihydrate Sigma](#)

Aldrich Catalog #E4884

[Iron\(III\) chloride hexahydrate Sigma](#)

Aldrich Catalog #44944

[Zinc sulfate heptahydrate Sigma](#)

Aldrich Catalog #204986

[Cobalt\(II\) chloride hexahydrate Sigma](#)

Aldrich Catalog #60820

[Manganese\(II\) chloride tetrahydrate Sigma](#)

Aldrich Catalog #M3634

[Sodium molybdate dihydrate Sigma](#)

Aldrich Catalog #M1651

[Sodium phosphate monobasic monohydrate Sigma](#)

Aldrich Catalog #S9638

[Instant Ocean aquarium sea salt mixture Contributed by](#)

users Catalog #SS15-10

[Sodium Orthovanadate Sigma](#)

Aldrich Catalog #S6508-10G

[Vitamin B12 Fisher](#)

Scientific Catalog #68-19-9

[100 ml Nickel Sulfate](#)

Hexahydrate biorbyt Catalog #orb65604

[Copper \(II\) sulfate pentahydrate Bio Basic](#)

Inc. Catalog #CDB0063.SIZE.2.5Kg

[Glucose Sigma](#)

Aldrich Catalog #G8270

Preparation of stock solutions

1 Stock solution A: Trace elements.

- Dissolve the following components in dH₂O to bring a final volume of 1 liter:

Na ₂ EDTA • 2H ₂ O	-----	4.36 g
FeCl ₃ • 6H ₂ O	-----	3.15 g
MnCl ₂ • 4H ₂ O	-----	178.1 mg
ZnSO ₄ • 7H ₂ O	-----	23 mg
CoCl ₂ • 6H ₂ O	-----	11.9 mg

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -----	2.5 mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ -----	19.9 mg
H_2SeO_3 -----	1.29 mg
$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ -----	2.63 mg
Na_3VO_4 -----	1.84 mg
K_2CrO_4 -----	1.94 mg

- Sterilize by filtration through 0.2 μm filter and store at 4 °C.

Stock solution B: Vitamins.

- Dissolve the following components in dH₂O to bring a final volume of 1 liter:

thiamine • HCl (vit. B ₁) -----	200 mg
biotin (vit. H) -----	1 mg
cyanocobalamin (vit. B ₁₂) -----	1 mg

It is recommended to prepare primary stock solutions of biotin (100 mg per liter) and cyanocobalamin (1 g per liter), and use, respectively, 10 and 1 ml to prepare 1 liter of stock solution B.

- Sterilize by autoclaving and store at 4 °C.

Stock solution C: Phosphate.

- Dissolve 5 mg of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 1 liter of dH₂O.
- Sterilize by filtration through 0.2 μm filter and store at 4 °C.

Base mL1 medium preparation (working solution)

- 2
 - Dissolve 35 g of marine salts (Instant Ocean) and 10 g of glucose in 900 ml of dH₂O.
 - Add 1 ml of Stock solution A (trace elements)
 - Add 40 μl of Stock solution B (vitamins)
 - Add 1 ml of Stock solution C (phosphate).
 - Bring to a final volume of 1 liter with dH₂O.
 - Sterilize by filtration through 0.2 μm filter and store at 4 °C.

IMPORTANT: The lack of nitrogen source in this medium is intentional. If the purpose is not to study nitrogen metabolism, it can be supplemented with 75 mg per liter of NaNO_3 or NH_4NO_3 .