VERSION 1

JUL 12, 2021

OPEN ACCESS

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>

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

Created: Jan 02, 2019

Last Modified: Jul 12, 2021

PROTOCOL integer ID:
18920

Preparation of defined medium for marine holozoans. V.1

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

Multicellgenomelab

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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>).

MATERIALS

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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

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ -----	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 dH_2O to bring a final volume of 1 liter:

thiamine $\cdot$ HCl (vit. B ₁) -----	200 mg
biotin (vit. H) -----	1 mg
cyanocobalamin (vit. B ₁₂) -----	1 mg

Note

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 dH_2O .
- Sterilize by filtration through 0.2 μm filter and store at 4 °C.

Base mL1 medium preparation (working solution)

- Dissolve 35 g of marine salts (Instant Ocean) and 10 g of glucose in 900 ml of dH_2O .
 - 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 dH_2O .
 - Sterilize by filtration through 0.2 μm filter and store at 4 °C.

Note

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 .