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Working

Preparation of defined medium for marine holozoans. [↗](#)

Version 2

PLOS Genetics

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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>).

EXTERNAL LINK

<https://doi.org/10.1371/journal.pgen.1007986>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

MATERIALS

NAME	CATALOG #	VENDOR
Biotin	View	P212121
Thiamine HCl	View	P212121
Potassium chromate		
Selenious acid	View	P212121
Ethylenediaminetetraacetic acid disodium salt dihydrate	E4884	Sigma Aldrich
Iron(III) chloride hexahydrate	44944	Sigma Aldrich
Zinc sulfate heptahydrate	204986	Sigma Aldrich
Cobalt(II) chloride hexahydrate	60820	Sigma Aldrich
Manganese(II) chloride tetrahydrate	M3634	Sigma Aldrich
Sodium molybdate dihydrate	M1651	Sigma Aldrich
Sodium phosphate monobasic monohydrate	S9638	Sigma Aldrich
Instant Ocean aquarium sea salt mixture	SS15-10	

NAME ▾	CATALOG # ▾	VENDOR ▾
Sodium Orthovanadate	S6508-10G	Sigma Aldrich
Vitamin B12	68-19-9	Fisher Scientific
100 ml Nickel Sulfate Hexahydrate	orb65604	biobyte
Copper (II) sulfate pentahydrate	CDB0063.SIZE.2.5Kg	Bio Basic Inc.
Glucose	G8270	Sigma Aldrich

Preparation of stock solutions

1 Stock solution A: Trace elements.

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

Na₂EDTA • 2H₂O ----- 4.36 g
 FeCl₃ • 6H₂O ----- 3.15 g
 MnCl₂ • 4H₂O ----- 178.1 mg
 ZnSO₄ • 7H₂O ----- 23 mg
 CoCl₂ • 6H₂O ----- 11.9 mg
 CuSO₄ • 5H₂O ----- 2.5 mg
 Na₂MoO₄ • 2H₂O ----- 19.9 mg
 H₂SeO₃ ----- 1.29 mg
 NiSO₄ • 6H₂O ----- 2.63 mg
 Na₃VO₄ ----- 1.84 mg
 K₂CrO₄ ----- 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. B₁) ----- 200 mg
 biotin (vit. H) ----- 1 mg
 cyanocobalamin (vit. B₁₂) ----- 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 g of NaH₂PO₄ • H₂O in 1 liter of dH₂O.
- 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 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 NaNO₃ or NH₄NO₃.



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