JUN 25, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.33xgqpn

Protocol Citation: Lev Tsypin, Aaron Turkewitz 2019. Media and reagents for *Seminavis robusta* cultivation and experiments.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.33xgqpn>

MANUSCRIPT CITATION:

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

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

Created: Jun 12, 2019

Last Modified: Jun 25, 2019

PROTOCOL integer ID:
 24407

Media and reagents for *Seminavis robusta* cultivation and experiments

In 2 collections

Lev Tsypin¹, Aaron Turkewitz²

¹California Institute of Technology; ²University of Chicago

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

Lev Tsypin

ABSTRACT

This protocol describes the culturing conditions used to grow *S. robusta* in the lab for all experiments.

GUIDELINES

It matters whether you use NanoPure/MilliQ water or glass distilled water. In glass distilled water-based media, *S. robusta* tends to disadhere from the bottom of the culture flask and float more readily than in MilliQ-based media.

Keywords: Diatom, marine, protist, cultivation

MATERIALS

MATERIALS

- ⊗ Bioactif artificial sea salt **Tropic Marin Catalog #10304**
- ⊗ Guillard's (F/2) Marine Water Enrichment Solution **Sigma Aldrich Catalog #G9903-500ML**
- ⊗ Sodium bicarbonate **Sigma Aldrich Catalog #S6014**
- ⊗ Penicillin G sodium salt **Sigma Aldrich Catalog #P3032-25MU**
- ⊗ Gentamycin sulfate **Sigma Aldrich Catalog #345814-1GM-M**
- ⊗ Ampicillin sodium salt **Fisher Scientific Catalog #BP1760-25**
- ⊗ Streptomycin sulfate **MP Biologicals Catalog #100556**
- ⊗ Agar **Fisher Scientific Catalog #BP1423-500**
- ⊗ 175 cm² flasks with sealing caps **Thermo Scientific Catalog #156502**
- ⊗ 25 cm² flasks with sealing caps **USA Scientific Catalog #CC7682-4325**
- ⊗ Stericup **Millipore Catalog #SCGPT05RE**
- ⊗ Cell Scraper **Fisher Scientific Catalog #08-100-241**
- ⊗ D-sorbitol **Sigma Aldrich Catalog #M4125**
- ⊗ Mannitol **Sigma Aldrich Catalog #S1876**

Preparation of F/2 medium

- 1 Dissolve 34.5 g Tropic-Marin Bioactif (TMB) in 1 L MilliQ water

- 2 Add 80 mg sodium bicarbonate

- 3 Autoclave

- 4 After cooling, add 20 mL 50x Guillard's supplement

5 Filter using Stericup into pre-sterilized bottles

6 Store at 4C in the dark

Maintenance antibiotics (200x stock)

7 Dissolve 1 g penicillin G sodium salt , 1 g ampicillin sodium salt ,
0.2 g streptomycin sulfate , and 0.1 g gentamycin sulfate in a final volume of
20 mL F/2 medium .

8 Aliquot and store at -20 C.

Agar plates

9 Dissolve 17.25 g TMB in 1 L MilliQ

10 Add 80 mg sodium bicarbonate

11 Add 0 µL 15 g agar , dissolve while stirring and heating (may require boiling)

- 12 Autoclave
- 13 After cooling below 60 C, add 20 mL 50x Guillard's supplement, as well as selective antibiotics, if desired.
- 14 Pour approx. 19 mL per plate
- 15 Store in the dark at 4 C

Electroporation buffers

- 16 1 Molarity (M) sorbitol in MilliQ, 0.77 Molarity (M) mannitol in MilliQ, or 0.77 Molarity (M) mannitol with 8 % volume F/2 in MilliQ.