

Sep 24, 2019

Hornwort growth media

DOI

dx.doi.org/10.17504/protocols.io.4x2gxqe



Eftychis Frangedakis¹

¹University of Cambridge



Eftychis Frangedakis

University of Cambridge, Plant Sciences

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.4x2gxqe

Protocol Citation: Eftychis Frangedakis 2019. Hornwort growth media . **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.4x2gxqe>

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: June 29, 2019

Last Modified: September 24, 2019

Protocol Integer ID: 25306

Abstract

Hornworts have a remarkable regenerative capacity. For tissue culturing / propagation you just need to transfer a small tissue fragment on fresh media and that's it... it will keep growing.



Materials

MATERIALS

 Duchefa Gelrite™ Gold Biotechnology Catalog #G1101

G1910 - Sigma

Gelzan™ CM

Gelrite®

Synonym: Agar substitute gelling agent, Gellan Gum

BCD medium

Stock B (1L)

25g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$

Stock C (1L)

25g KH_2PO_4

pH to 6.5 with KOH

Stock D (1L)

101g KNO_3

1.25g $\text{FeSO}_4 \times 7\text{H}_2\text{O}$

Stock CaCl_2 (1L)

14.7g $\text{CaCl}_2 \times 2\text{H}_2\text{O}$

Trace element solution (1L)

55mg $\text{CuSO}_4 \times 5\text{H}_2\text{O}$

614mg H_3BO_3

55mg $\text{CoCl} \times 6\text{H}_2\text{O}$

25mg $\text{NaMoO}_4 \times 2\text{H}_2\text{O}$

55mg $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$

389mg $\text{MnCl}_2 \times 4\text{H}_2\text{O}$

28mg KI

BCD working solution (1L)

10ml Stock B

10ml Stock C

10ml Stock D

10ml Stock CaCl_2



1ml Trace element solution

[for plate: 8g agar]

KNOP

Stock 1 (1L, autoclave)

25g KH_2PO_4

Stock 2 (1L, autoclave)

25g KCl

Stock 3 (1L, autoclave)

25g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$

Stock 4 (1L, autoclave)

100g $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$

KNOP working solution (1L, autoclave)

10mL Stock 1

10mL Stock 2

10mL Stock 3

10mL Stock 4

12.5mg $\text{FeSO}_4 \times 7\text{H}_2\text{O}$

pH to 5.8 with KOH

- 1 Using a sterile scalpel transfer a small thallus tissue fragment (around 3mm x 3mm, but can also be smaller or bigger) on KNOP or BCD fresh media plates. Grow at 21°C, 12 h of light and 12 h of dark, 1500 lux light intensity
- 2 Subculture every 1-2 months

3

