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Hornwort sporophyte induction - Bonn

Eftychis Frangedakis[1]

[1]University of Cambridge

1 Works for me dx.doi.org/10.17504/protocols.io.4x5gxq6

Eftychis Frangedakis
University of Cambridge, Plant Sciences , OpenPlant

EXTERNAL LINK

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MATERIALS TEXT

G1910 - Sigma
Gelzan™ CM
Gelrite®
Synonym: Agar substitute gelling agent, Gellan Gum

BCD medium

Stock B (1L)
25g $MgSO_4$ x $7H_2O$

Stock C (1L)
25g KH_2PO_4
pH to 6.5 with KOH

Stock D (1L)
101g KNO_3
1.25g $FeSO_4$ x $7H_2O$

Stock $CaCl_2$ (1L)
14.7g $CaCl_2$ x $2H_2O$

Trace element solution (1L)
55mg $CuSO_4$ x $5H_2O$
614mg H_3BO_3
55mg CoCl x $6H_2O$
25mg $NaMoO_4$ x $2H_2O$
55mg $ZnSO_4$ x $7H_2O$
389mg $MnCl_2$ x $4H_2O$
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]

1 Grow small thallus fragments for two weeks in petri dishes on Knop or BCD media at pH 5.7 and containing 0.7% (w/v) Gelzan (A in figure).

2 Transfer plants to Magenta pots on Knop medium or BCD medium (B and C in figure) at pH 5.7, containing 0.7% (w/v) Gelzan. Add 2mL of sterile water into the pot using a pipette.

3 Place pots in a Panasonic MLR-352 Versatile Environmental Test Chamber (or similar growth chamber / tissue cluture room) at 21^{O}C, 12 h of light and 12 h of dark, 1500 lux light intensity.

4 After approximately one month, antheridia start to appear. Add another 1-2 mL of sterile water into the pot using a pipette (D in figure).

5 After one more month sporophytes emerge (E in figure).

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