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 We use this protocol and it's working

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🌐 Hornwort sporophyte induction - Bonn

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MATERIALS

G1910 - Sigma

Gelzan™ CM

Gelrite®

Synonym: Agar substitute gelling agent, Gellan Gum

BCD medium

Stock B (1L)

25g MgSO₄ x 7H₂O

Stock C (1L)

25g KH₂PO₄

pH to 6.5 with KOH

Stock D (1L)

101g KNO₃

1.25g FeSO₄ x 7H₂O

Stock CaCl₂ (1L)

14.7g CaCl₂ x 2H₂O

Trace element solution (1L)

55mg CuSO₄ x 5H₂O

614mg H₃BO₃

55mg CoCl x 6H₂O

25mg NaMoO₄ x 2H₂O

55mg ZnSO₄ x 7H₂O

389mg MnCl₂ x 4H₂O

28mg KI

BCD working solution (1L)

10ml Stock B

10ml Stock C

10ml Stock D

10ml Stock CaCl₂

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 culture room) at 21°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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