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 We use this protocol and it's working

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🌐 Hornwort growth media

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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

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Duchefa Gelrite™ Gold Biotechnology Catalog
 #G1101

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