Oct 27, 2020

Culturing *Physcomitrella patens*

Igem Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.bn2rmgd6

Igem Dusseldorf

DOI

dx.doi.org/10.17504/protocols.io.bn2rmgd6

PROTOCOL CITATION

Igem Dusseldorf 2020. Culturing Physcomitrella patens. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bn2rmgd6

CREATED

Oct 27, 2020

LAST MODIFIED

Oct 27, 2020

PROTOCOL INTEGER ID

43825

GUIDELINES

All solutions and materials must be sterile.
$MgSO_4 \times 7\ H_2O$, $FeSO_4 \times 7\ H_2O$ and antiobiotics stock solutions must be sterilized **only** via filtration. All other solutions can be autoclaved.
Cellophane disks should be divided by simple paper, e.g. printing paper, for autoclaving to avoid them sticking together.
The whole culturing operation must be carried out under a horizontal laminar flow hood to assure sterile conditions.

Petri dishes should be sealed off in a way that still allows gas exchange.

MATERIALS TEXT

Macro elements:
- 0,8 g/L calcium nitrate tetrahydrate – $CaNO_3 \times 4\ H_2O$
- 0,25 g/L magnesium sulfate heptahydrate – $MgSO_4 \times 7\ H_2O$
- 0,0125 g/L iron(II) sulfate heptahydrate – $FeSO_4 \times 7\ H_2O$

Make a stock solution at 100 times the given concentration for each macro element.

Micro elements:
- 0,055 mg/L copper(II) sulfate pentahydrate – $CuSO_4 \times 5\ H_2O$
- 0,055 mg/L zinc(II) sulfate heptahydrate – $ZnSO_4 \times 7\ H_2O$
- 0,614 mg/L orthoboric acid – H_3BO_3
- 0,389 mg/L manganese(II) chloride tetrahydrate – $MnCl_2 \times 4\ H_2O$
- 0,055 mg/L cobalt(II) chloride hexahydrate – $CoCl_2 \times 6\ H_2O$

■ 0,028 mg/L potassium iodide – KI
■ 0,025 mg/L sodium molybdate dihydrate – $Na_2MoO_4 \times 2\ H_2O$

Make a stock solution at 1000 times the given concentration for each micro element.

Other elements:
■ 250 mg/L monopotassium phosphate – KH_2PO_4

Put 25 g in 100 mL water, titrate with potassium hydroxide (KOH) to pH 7 for a stock solution at 1000 times the given concentration.
■ 500 mg/L ammonium tartrate – $(NH_4)2C_4H_4O_6$

Put 18,415 g in 100 mL water for a 1 M sock solution.

Other materials:
■ 90 mm sterile petri dishes
■ Cellophane disks:
Prepare cellophane sheets and cut with a tool to the appropriate size for the petri dishes.
■ 3 MTM Micropore surgical tape or any equivalent
■ Spatula
■ Homogenizer
■ Common tools for making solid agar

DISCLAIMER:

Prepare in advance

1 Liquid PPNH4 medium with 300 mg/L Cefotaxim or 250 mg/L Vancomycin.

1.1 Add 10 mL of a macro element-solution, 1 mL of a micro element solution, 1 mL of the potassium phosphate solution and 2,7 mL of the ammonium tartrate solution for each liter of medium.

2 Solid PPNH4 medium on sterile petri dishes

2.1 Add 7,2 g agar per liter of PPNH4-liquid medium. Add further 300 mg cefotaxime or 250 mg Vancomycin per liter of
medium to suppress bacterial growth.

3 Cellophane disks with the appropriate size for the petri dishes. Wet the cellophane disks with sterile ultrapure water to avoid creases.

3.1 Overlay the solid medium on the petri dishes with fitting cellophane disks to prevent the mosses from growing into the medium.

Citation: Igem Dusseldorf (10/27/2020). Culturing Physcomitrella patens. https://dx.doi.org/10.17504/protocols.io.bn2rmgd6

Execution

4 Scrape 7 – 10 days old moss protonemal tissue with spatula.

5 Put 4 mL of water into a (conical tube). Put in the protonemal tissue. Cut it with a Homogenizer until no big chunk is visible and the water is uniformly green.

6 Spread 2-3 mL of freshly fragmented protonema on the cellophane on the petri dish.

7 Seal off with micropore tape to allow gas exchange.

8 Incubate plates at 24°C with a light regime of 16 h light : 8 h darkness at 80 µmol×m–2×s–1

9 For next harvest gently scrape surface of cellophane disks after 7 – 10 days (before culture starts to turn brown). Repeat cycle. PPNH4 liquid medium with 300 mg/L Cefotaxim or 250 mg/L Vancomycin is recommended for storage as the moss stays available for culture for >6 months.

Citation: Igem Dusseldorf (10/27/2020). Culturing Physcomitrella patens. https://dx.doi.org/10.17504/protocols.io.bn2rmgd6