

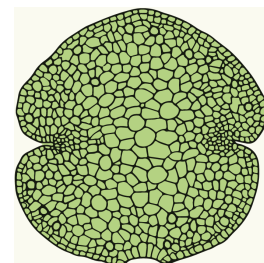
Dec 15, 2025

Version 2

Marchantia protoplast V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge6z6wl47/v2>



Eftychis Frangedakis¹

¹University of Cambridge

Hornworts



Eftychis Frangedakis

University of Cambridge, Plant Sciences

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.261ge6z6wl47/v2>

Protocol Citation: Eftychis Frangedakis 2025. Marchantia protoplast. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.261ge6z6wl47/v2> Version created by [Eftychis Frangedakis](#)

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: December 15, 2025

Last Modified: December 15, 2025

Protocol Integer ID: 235090

Keywords: marchantia protoplast protocol for the preparation, marchantia protoplast protocol

Abstract

Protocol for the preparation of *Marchantia polymorpha* protoplasts

Materials

8% (w/v) mannitol at pH 5.7

Solutions/media to prepare before starting the protocol (Day 1 & Day 2):

1 Day 1

8% (w/v) mannitol at pH 5.7

Liquid Gamborg B5 plus vitamins

- 0.0948 g Gamborg B5 plus vitamins
- 2.4g mannitol
- Adjust pH to 5.7
- dH₂O to 29.7 ml

Day 2

Protoplast Regeneration Media Top layer (PRMT)

- 0.0948 g Gamborg B5 plus vitamins
- 1.6 g mannitol
- 0.1 g agar
- dH₂O to make up to 19.6 ml
- Adjust pH to 5.7
- Autoclave and keep at 50°C after autoclaving.

Protoplast Regeneration Media Bottom layer (PRMB)

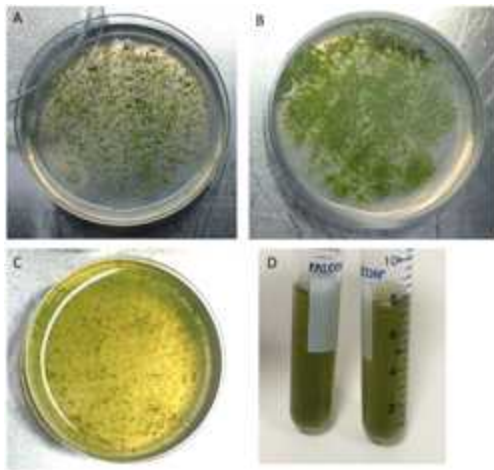
- 1.57 g Gamborg B5 plus vitamins
- 30 g mannitol
- Adjust pH to 5.7
- 4 g agar
- dH₂O to make up to 495 ml
- Autoclave - Keep at 50 °C after autoclaving, and add 5ml sterile 50% glucose before use as indicated in the protocol.

Gemmae growth (Day -4)

2

Grow gemmae on ½ Gamborg B5 plus vitamins media plates (the plates should be covered like Fig. 1A) for 4 days (Fig. 1B).

2.1

**Figure 1**

Day 1

- 3 Add 10 ml of 8% (w/v) mannitol at pH 5.7 on the plate and incubate at room temperature 30min.

Day 1

- 4 Prepare 2% Driselase solution: add 0.2 g Driselase in 10 ml 8% mannitol solution and incubate in dark for 20 min at RT. Gently invert to mix at intervals. Spin at 3.3k for 3 min. Filter sterilize supernatant using a 0.2 μ m filter attached to a syringe.
- 5 Replace mannitol solution with 5 ml 2% Driselase and incubate at RT for 4 hours with gentle shaking (50 rpm).
- 6 Using a tip gently release protoplasts from gemmae ("dissolve the gemmae") (Fig.1C).
- 7 Filter the solution through a 70 μ m cell strainer into a 50 ml falcon tube and then transfer in a round bottom falcon tube (Fig. 1D).
- 8 Incubate for further 10 min.



- 9 Spin at 120 x g for 3min. Remove the supernatant without disturbing the cells.
- 10 Wash the cells by re-suspending them in 6ml 8% mannitol (Mix by swirling not shaking) and spin at 120 x g for 3 min. Repeat the wash 2 times.
- 11 Add 300 μ l 50% glucose in the autoclaved liquid Gamborg B5 plus vitamins + mannitol solution.
- 12 Wrap tubes in foil to keep them dark and leave them at 25C o/n.

Day 2

- 13 Prepare PRMB and PRMT and autoclave the media. After autoclaving, keep PRMT at 50°C.
- 14 Add glucose to PRMB and pour it in 90mm plates.
- 15 Spin cells down 120 x g for 4 min. Mix by swirling not shaking.
- 16 Remove the supernatant and gently re-suspend each pellet in 0.5 ml 8% mannitol if plating out on 3 plates, or in 0.8 ml if using 4 plates. Use mannitol from the 10ml 8% mannitol that is autoclaved separately.
- 17 Add 2.5ml PRMT to each cell suspension (that is sufficient for plating out on 3 plates). Quickly but gently mix by pipetting up and down and plate 1ml on each PRMB plate.