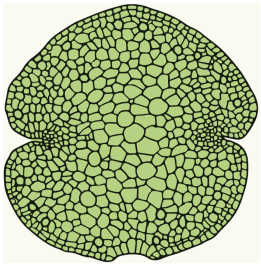
Dec 15, 2025 Version 2

Marchantia protoplast V.2



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Protocol status: Working
We use this protocol and it's working

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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
- dH_2O 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
- dH_2O to make up to 19.6 ml
- Adjust pH to 5.7
- Autoclave and keep at 50^{o}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
- dH_2O to make up to 495 ml
- Autoclave - Keep at 50 ^{o}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

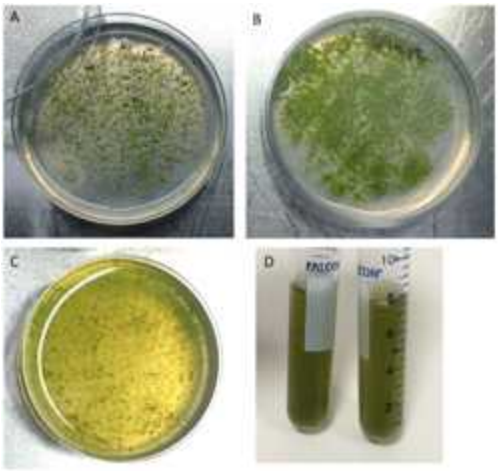

Figure 1

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 µm 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 µm 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 themin 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 µl 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.