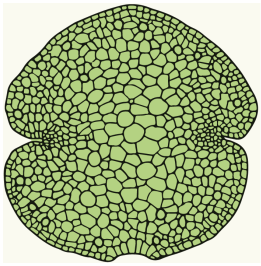
Sep 24, 2019

Marchantia protoplast



DOI
dx.doi.org/10.17504/protocols.io.5kag4se

Eftychis Frangedakis[1]

[1]University of Cambridge

OpenPlant Project

Eftychis Frangedakis
University of Cambridge, Plant Sciences

DOI: dx.doi.org/10.17504/protocols.io.5kag4se

Protocol Citation: Eftychis Frangedakis 2019. Marchantia protoplast. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.5kag4se**

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

Created: July 17, 2019

Last Modified: September 24, 2019

Protocol Integer ID: 25954

Materials

8% (w/v) mannitol at pH 5.7

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

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

3 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.

4 Replace mannitol solution with 5 ml *2%* Driselase and incubate at RT for 4 hours with gentle shaking (50 rpm).

5 Using a tip gently release protoblasts from gemmae (Fig.1C).

6 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).

7 Incubate for further 10 min.

8 Spin at 120 x g for 3min. Remove thesupernatant without disturbing the cells.

9 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.

10 Add 300 µl 50% glucose in the autoclaved liquid Gamborg B5 plus vitamins + mannitol solution

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