

Marchantia Protoplast Isolation

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Abstract

Marchantia protoplast preparation (adapted from Bopp and Vicktor 1988)

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Before start

Perform surface sterilization of Marchantia spores (see Step 1-7 in protocol “Agrobacterium mediated transformation of Marchantia spores” by Dr. Susana Sauret-Gueto). Let sporelings grow for 10-14 days post surface sterilization.

Protocol

Step 1.

Solution 1 (S1): dissolve 6% (w/v) Mannitol in half-strength Gamborg's B5 medium and filter sterilize.

Step 2.

Solution 2 (S2): dissolve 2% (w/v) Driselase and 6% (w/v) Mannitol in half-strength Gamborg's B5 medium and filter sterilize.

Step 3.

Pour S1 directly on agar plate containing 10-14 day old Marchantia sporelings and incubate for 30min at room temperature without agitation.

Step 4.

Carefully pour S1 off the agar plate and replace by S2. Ensure all sporelings are covered in S2. Incubate in the dark at room temperature for 2-10 hours without agitation.

Step 5.

Gently shake agar plate for 10 seconds to detach protoplasts.

Step 6.

Pour protoplast-containing S2 through 40 μ M Nylon mesh to remove debris.

Step 7.