ABSTRACT

This protocol is a modification of the ISOLATION OF MAIZE CHLOROPLASTS FOR PROTEIN IMPORT STUDIES protocol from Mark Settles (modified from Ken Cline).

GUIDELINES

*Pre-cool o/n Buffer GR and pestle and mortar.

MATERIALS

- **GR Buffer (1L)**
  - 50 mM Hepes-KOH pH7.5
  - 0.33 M Sorbitol
  - 1 mM MgCl2
  - 1 mM MnCl2
  - 2 mM EDTA

- 5 mM Na-ascorbate
- 1% BSA

**GR Buffer (1L)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration/Volume</th>
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<tbody>
<tr>
<td>Hepes-KOH</td>
<td>50 ml 1 M Hepes (23.8g/100ml) [238.3 - Melford]</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>100ml 3.3 M Sorbitol (300g/500ml) [182.2 - Melford]</td>
</tr>
<tr>
<td>MgCl2</td>
<td>1ml 1M MgCl2</td>
</tr>
<tr>
<td>MnCl2</td>
<td>1ml 1M MnCl2 (19.7g/100ml) [197.91 - Sigma]</td>
</tr>
<tr>
<td>EDTA</td>
<td>4ml 0.5M EDTA pH8</td>
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</table>

add fresh

- 5 mM Na-ascorbate
- 1 g Na-ascorbate [Acros Organic]
- 1% BSA
- 10g BSA
PROTOCOL integer ID:
25310

Keywords: marchantia
chloroplast isolation

1 Prepare 20 ml 30% Percoll solution by mixing 6ml Percoll, 14ml Buffer GR.

2 Prepare 10 ml 70% Percoll solution by mixing 7ml Percoll and 3ml Buffer GR.

3 Add 15ml of 30% Percoil solution in a 50ml Falcon tube. Very carefully underlay 6ml of 70%
(denser) Percoll solution using a 5ml Gilson pipette (A in Figure).

4 Keep the gradient on ice until further use.

5 Weigh 10g of thallus (grown in 12h light : 12h dark cycle – harvest the tissue 2-3 hours at the
start of the light cycle - to reduce starch accumulation) (B in Figure).

6 Homogenize the tissue using a mortar and pestle into 100ml buffer GR (C and D in Figure).

7 Filter homogenate through two layers of Miracloth into two 50ml falcon tubes (E and F in Figure).

8 Centrifuge at 1200 g for 7 min (G in Figure)
9. Remove supernatant and resuspend the pellet carefully in 2ml of buffer GR.

10. Transfer the resuspended pellet to the top of the Percoll Gradient using a cut-off pipette tip (H in Figure).

11. Spin at 7000g for 17 min at 4°C. USE A SLOW ACCELERATION AND TURN DECELERATION OFF. Broken chloroplasts will reside in the top fraction, intact chloroplasts will reside in the interface of the two Percoll layers (I in Figure).

12. Carefully transfer intact chloroplasts from the interface, to a new 50 mL Falcon tube.

13. Wash intact chloroplasts with 25ml of Buffer GR (without BSA). This step removes residual Percoll. Centrifuge at 1500g for 5min at 4°C (J in Figure).

14. Flash freeze pellet if not used immediately and store at -80°C