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Marchantia chloroplast isolation

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1 Works for me dx.doi.org/10.17504/protocols.io.4x6gxre

OpenPlant Project



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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

NAME	CATALOG #	VENDOR
Percoll™ Plus	17-5445-01	Ge Healthcare
Miracloth	475855	Merck Millipore

MATERIALS TEXT

GR Buffer (1L)

50 mM Hepes-KOH pH7.5	50 ml 1 M Hepes (23.8g/100ml) [238.3 - Melford]
0.33 M Sorbitol	100ml 3.3 M Sorbitol (300g/500ml) [182.2 - Melford]
1 mM MgCl ₂	1ml 1M MgCl ₂
1 mM MnCl ₂	1ml 1M MnCl ₂ (19.7g/100ml) [197.91 - Sigma]
2 mM EDTA	4ml 0.5M EDTA pH8

add fresh

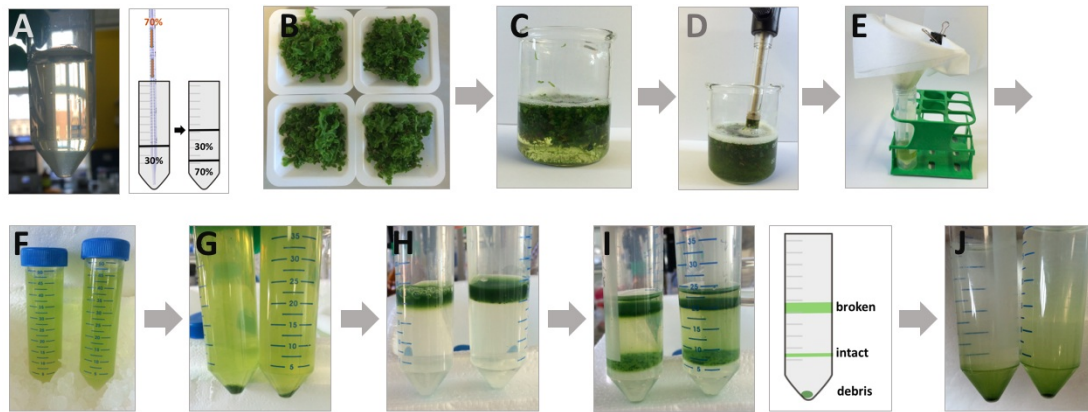
5 mM Na-ascorbate 1 g Na-ascorbate [Acros Organic]

1% BSA 10g BSA

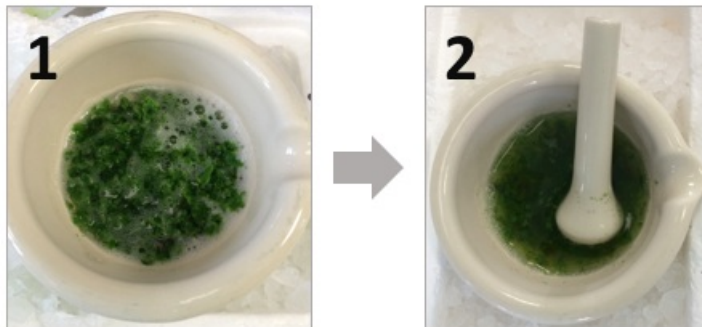
- 1 Prepare 20 ml 30% Percoll solution by mixing 6ml Percoll, 14ml Buffer GR.
- 2 Prepare 10 ml 70% Percoll solution by mixing 7ml Percoll and 3ml Buffer GR.
- 3 Add 15ml of 30% Percoll solution in a 50ml Falcon tube. Very carefully underlay 6ml of 70% (denser) Percoll solution using a 5ml Gilson pipette (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

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