Minute™ Chloroplast Isolation kit for Mucilaginous Plants

Catalog# CF-053

Description

Many crop species across the plant family contain high polysaccharide and polyphenols. The hydrocolloid mucilage is a complex mucopolysaccharide typically showing high viscosity when released in solution. Homogenate of high mucopolysaccharide plant samples usually imparts a slimy consistency that is most unwanted contaminants that interfere with cell fractionation and organelle purification, such as chloroplast and nuclear isolation. This kit provides a simple, rapid, and efficient method for isolating chloroplasts from viscous plant homogenate. The protocol takes about 30 min.

Kit Components (20 preps):

1.	Buffer A	30 ml
2.	Buffer B	30 ml
3.	Micro puncher	2

Additional Materials Required but Not Provided

- Table-top microcentrifuge
- 1.5ml Eppendorf tubes.

Shipping and Storage: Ship at ambient temperature and store at 4°C.

Protocol

Note: Read the protocol carefully before starting. Chill buffers on ice prior to use. *Perform all centrifugation steps at room temperature.*

1. Weight out 140-160mg of fresh young green plant leaves, fold and insert them into a 1.5 ml microfuge tube. Press them down to the bottom using the larger opening end of a 200 µl pipette tip. Add 200 µl buffer A and gently punch the sample about 400-500 times (~3 min) with a micro puncher provided, turning the sample into a viscous slurry.

2. Add 0.8 ml buffer A to the tube while the micro puncher is still in the tube. Continue to punch the sample about 100 times to mix. In most cases, the homogenate still shows moderate viscosity.
   The puncher is reusable - rinse with water and dry with a paper towel.

3. Cap the tube and centrifuge at 8,000 X g for 5 min (see tech notes below). Decant and drain the viscous supernatant by gravity after centrifugation.
   The pellet contains two parts: the large debris (the lower portion); and separated intact chloroplasts (on the top).

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4. Slowly add 0.9 ml buffer B, and pipette up and down gently 15-20 times using a 1 ml pipette tip without touching the green pellet (this is to wash the chloroplasts out from the top of the pellet). With the progression of the pipetting, the clear supernatant will turn somewhat greenish. Transfer the supernatant to a fresh 1.5 ml Eppendorf tube.

5. Centrifuge at 5,000 X g for 5 min. A green pellet should be visible. Remove all supernatant with a 1ml pipette tip. If a gel-like layer is visible on top of the green pellet, pipette up and down gently to resuspend this layer without disturbing the green pellet and discard the supernatant. Resuspend the pellet in 0.5 ml of the buffer B. Centrifuge at 2,000 X g for 5 min. The green pellet contains isolated chloroplasts.

6. Isolated chloroplasts can be resuspended in 100-400 µl buffer A or a buffer of your choice, depending upon downstream experiments. For protein analysis see the table below. BCA assay is recommended for protein concentration determination. The chloroplasts can also be used for nucleic acid extraction.

Tech Notes:

1. This kit is specially designed for plants with viscous homogenates, such as leaves of strawberries, water spinach, sweet potatoes, cherry, and plum tree. Don't use more than 160 mg of starting material. For Non-viscous plant samples, please use Minute™ Chloroplast Isolation Kit (Cat# CP-011).

2. Reduce the amount of starting material if the supernatant is too viscous to decant in step 3.

3. The centrifugal force in step 3 may vary. 8,000 X g should work well for most samples. However, the centrifugation force can be reduced to 5,000 X g if the samples are less viscous or the chloroplast yield is low (much smaller pellet).

Following reagents are recommended for solubilization of isolated chloroplasts

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.