Minute™ Chloroplast Isolation Kit

Catalog number: CP-011

Description

Invent Biotechnologies Minute™ chloroplast isolation kit is composed of optimized chloroplast isolation buffers and filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly isolate intact chloroplasts from fresh plant tissues (leaves, seeds and soft stems etc.). Due to the use of filter cartridges with pre-defined pore size and thickness, intact chloroplasts can be isolated from 50-200 mg fresh plant tissues in less than 5 min. Unlike many other methods that require 1-10+ gram tissues for chloroplast isolation, this kit can quickly obtain 1 X 10^6 to 1 X 10^7 intact chloroplasts (>90% intact) from fresh plant leaves.

Application

Minute™ chloroplast isolation kit is designed to rapidly isolate intact chloroplasts from small samples (50-200 mg) of fresh plant tissues for applications such as biochemical analysis, SDS-PAGE, immunoblotting, enzyme assays etc. Isolated chloroplasts can be used as starting materials for purification of RNA and DNA for molecular biology studies.

Kit components(50T):

1. 25 ml buffer A
2. 25 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. Plastic rod X 2

Kit components(4T):

1. 2.0 ml buffer A
2. 2.0 ml buffer B
3. 4 protein extraction filter cartridges
4. 4 collection tubes with cap
5. Plastic rod X 1

Storage: Store the kit at -20°C

Additional Materials Required

Table-Top Microcentrifuge, 1X PBS or 1X TES buffer

Chloroplast Isolation Procedures

Following procedures are for isolation of intact chloroplasts from 100-200 mg fresh plant tissue samples (leaves, seeds and soft stems etc.). If smaller or larger amounts of starting materials are used adjust the amount of buffer A proportionately. Pre-chill buffers and the filter cartridge in collection tube on ice.

1. Place 100-200 mg fresh plant tissue in the filter. **For plant leaf**, fold or roll the leaf and insert it into the filter. Punch the leaf in the filter repeatedly with a 200 µl pipette tip for about 100 times to reduce the volume.
2. Add 100 µl cold buffer A **(shake the bottle vigorously for a few times prior to pipetting)** to the filter. Grind the tissue with a plastic rod provided for about 100 times (about 2-3 min, note: the plastic rod is reusable. For cleaning, rinse it with water and dry with paper towel). Add another 300 µl buffer A to the filter and mix by stirring with the pipette tip.
3. Cap the filter and centrifuge in a microcentrifuge at 2,000 X g at 4°C for 4 min. Discard the filter, remove the supernatant in collection tube and resuspend the pellet in 500 µl cold buffer B by pipetting up and down or vortexing.

4. Centrifuge at 2,000 X g for 5 min at 4°C. Remove the supernatant and save the pellet (this is isolated chloroplasts). Resuspend the pellet including the chloroplasts adhere to the side wall of the tube in 100 to 200 ul of PBS (phosphate buffered saline),TES (2-[Tris(hydroxymethyl)-methylamino]-ethanesulfonic acid physiological buffer) or buffer of your choice depending upon specific downstream applications. Reagents in following table is recommended for solubilization of the pellet. For isoelectric focusing (First dimension of 2D gel) we recommend to use: 7M urea/2M thio-urea/2% Chaps and 20 mM DTT (add DTT to above mix prior to use).

Optional Further Cleanup of Isolated Chloroplasts

For most samples, above protocol will generate chloroplasts clean enough for most downstream applications. However, for some samples, if excessive debris is a concern, Percoll (Sigma, P-1644) can be used for further cleanup:

1. To a 1.5 ml microfuge tube add 0.3 ml Percoll and 0.7 ml cold 1 X PBS or TES, mix by vortexing for 10-20 seconds.
2. Resuspend chloroplast pellet from step 4 in 100 ul 1 X PBS or TES and carefully overlay on the top of diluted Percoll solution. And centrifuge at 11,000 X g for 10 min at 4°C.
3. Remove supernatant and resuspend the green pellet at the bottom of the tube in 100 µl PBS or TES and transfer to a fresh tube (Don't wash light green material on the side wall of the tube). The isolated chloroplast can be washed with 0.5 ml cold PBS to remove residue Percoll.

Following protein solubilization reagents are recommended.

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.