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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 (100-200 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

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

Storage: Store the kit at -20°C

Additional Materials Required

Table-Top Microcentrifuge with a maximum g force of 14,000-16,000.
1X PBS or 1X TES buffer

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Chloroplast Isolation Procedures

Following procedures are for isolation of intact chloroplasts from 50-200 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 50-200 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 1 ml pipette tip for 60-70 times to reduce the volume.
2. Add 200 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 50-60 times (about 2 min, note: the plastic rod is reusable. For cleaning, rinse it with water and dry with paper towel). Add another 100-200 µl buffer A to the filter and mix by stirring with the pipette tip.
3. Cap the filter and centrifuge in a microcentrifuge at 2000 X g at 4°C for 2-3 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 2000 X g for 2-3 min at 4°C. Remove the supernatant and save the pellet (this is isolated chloroplasts). The chloroplast pellet can be resuspended in any buffers 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).

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.