Minute™ Plant Golgi Apparatus Enrichment Kit

Catalog number: PG-049

Description

The plant Golgi apparatus plays a critical role in the biosynthesis of cellular structural components and intracellular protein trafficking. However, proteomic characterization of the Golgi has been limited by the lack of efficient methods for obtaining enriched Golgi fractions. Traditional Golgi isolation methods rely heavily on harsh homogenization and density-gradient centrifugation, which are labor-intensive, time-consuming, and typically require large amounts of starting material.

This kit is designed to overcome these limitations by combining gentle spin column-based homogenization, differential centrifugation, and selective precipitation to enrich Golgi apparatus from plant tissues. The protocol is simple, rapid, and straightforward, requiring only milligram quantities of starting material.

Using this approach, users can achieve a 2–3-fold enrichment of the Golgi fraction while better preserving the structural integrity of the isolated Golgi apparatus than traditional isolation methods. This makes the kit well suited for downstream biochemical, proteomic, and functional analyses.

Kit Components (20 preps):

Buffer A	10 ml
Buffer B	1.0 ml
Buffer C	4 ml
Buffer D	0.5 ml
Plastic rods	2
Filter Cartridge	20
Collection Tube	20

Important Note

1. All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated microcentrifuge. Add proteinase/phosphatase (if protein phosphorylation is involved) inhibitor cocktails to aliquot of buffer A.
2. Chill buffers on ice prior to use.
3. Solution required but not provided: cold dd H_2O.

Protocol

1. Place 200-250 mg fresh plant leaf /seedling in the filter with collection tube. Fold and roll the leaf and insert it into the filter. Add 100 µl buffer A to the filter**.** Punch the leaf in the filter repeatedly with a 200 µl pipette tip for about 100-200 times to reduce the volume (this step takes about 2-3 min).
2. Grind the tissue with the plastic rod provided using gentle twisting force for about 200 times (about 2-3 min). (Note: the rod is reusable. For cleaning, rinse it with dd H_2O and dry with paper towel).

3. Add 400 µl buffer A to the filter and stir the sample with a 200 µl pipette tip for a few times. Cap the filter and Centrifuged at 5,000 X g for 10 min. After centrifugation, transfer 500 µl supernatant to a fresh 1.5 ml microfuge tube.
4. Add 50 µl buffer B to the tube, vortex briefly to mix well and incubate on ice for 30-40 min.
5. Centrifuge at 11,000 X g for 10 min. Remove and discard the supernatant completely. Add 1 ml cold ddH_2O to the tube without disturbing the pellet. Remove the water immediately. This is to remove excessive rubisco on the wall of the tube
6. Resuspend the pellet in 150-200 µl buffer C by pipetting up and down for 30-40 times. Incubate on ice for 15 min. Vortex every 5 min. Centrifuge the tube at 5,000 X g for 5 min to remove aggregated materials (usually seen as a light-green pellet).
7. Transfer supernatant to a fresh microfuge tube and mix with 1/10th volume of buffer D (for example mix 20 µl buffer D with 200 µl supernatant). The final mix is enriched Golgi fraction. Protein concentration can be assayed directly using BCA kit (Pierce). Typically, the protein yield is 40-60 µg/sample.
8. If the prep is not used right away, store it at -80ºC. Check the enrichment of Golgi by specific marker antibody and use total tissue lysate as an internal control in Western blotting. Make sure that equal protein loading in the gel. Isolated Golgi fraction can be used directly in SDS-PAGE and Western blotting after mix with proper amount of loading buffer. For other applications see table below.

Tech Note

1. This kit has been tested on leaves of *A. thaliana, N. tabacum, B. rapa and B. napus*. Other sample type may be used but the performance of the kit is sample-type dependent.
2. The degree of Golgi enrichment is also sample type-dependent. Generally, 2-3 folds of enrichment can be expected for most samples.
3. If protein concentration in isolated Golgi fraction is low (step 7), the pellet in step 6 can be resuspended in 100 µl buffer C. However this may cause some protein loss.
4. Isolated Golgi may show certain degree of cross-contamination by ER depending upon tissue types.

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.