# Minute™ Plant ER Enrichment Kit

Catalog number: PR-048

## Introduction

Isolation and enrichment of plant endoplasmic reticulum (ER) is essential for elucidation of ER related cellular functions. Traditional method for ER isolation is tedious and time consuming. In animal cells, ER is closely associated microtubules and Golgi apparatus are clustered at microtubule organizing center. In plant cells, the ER is associated with actin microfilaments and no microtubule organizing center is found near the nuclei. Due to structural characteristics of plan ER, it is much more difficult to isolate/enrich plant ER than that of animal samples. Minute™ plant ER enrichment kit was specially designed for plants. One to three-fold enrichment of ER fraction can be obtained from plant leave samples in about 1 hour. The enriched ER is essentially free from Golgi apparatus contamination. The enriched ER fraction can be used in plant protein trafficking studies.

## Kit Components (20 prep)

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| Buffer A | 20 ml |
| Buffer B | 1.2 ml |
| Buffer C | 10 ml |
| Plastic Rods | 2 |
| Filter Cartridge | 20 |
| Collection Tube | 20 |

## Important Note

1. Read the protocol carefully prior to experiment. All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated microcentrifuge. Add proteinase/phosphatase inhibitor cocktail to aliquot of buffer A if protein phosphorylation is involved in the study.
2. Chill buffer on ice prior to use
3. Solutions needed but not provided: cold ddH2O

## Protocol

1. Place 200-250 mg fresh plant leaf/young seedlings in the filter with collection tube. Fold and insert it into the bottom of 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 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 H2O and dry with paper towel). 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 6000 X g for 5 min. Remove the filter and resuspend the pellet by vortexing. Centrifuge at 16,000 X g for 15 min. pour out the supernatant.

3. Add 1.5 ml cold ddH2O carefully to the tube without disturbing the pellet. Immediately remove and discard the supernatant completely.
4. Add 450 µl cold buffer A and 50 ul buffer B to the tube. Pipette up and down repeatedly to resuspend the pellet (takes about 2 min). Vortex the tube vigorously for 20 seconds. Incubate on ice for 5 min and centrifuge at 10,000 X g for 10 min. Transfer all supernatant to a fresh 1.5 ml tube.
5. Add 0.5 ml buffer C to the tube, mix well and incubate on ice for 10 min. Centrifuge at 16,000 X g for 10 min. Remove supernatant completely and spin at 16,000 X g for 2 min to bring down residual reagent. Remove the reagent completely. The pellet is enriched ER fraction
6. Resuspend the pellet in 100-200 ul buffer of choice (depending upon downstream applications). The protein concentration can be determined by BCA assay (Pierce). The total protein is usually in the range of 40-80 µg/sample. Protein solubilization reagents in the table below are recommended for related applications.

**Tech Note:**

1. This protocol has been tested using plant leaves, other sample type such as seed and soft stems may also be used but the performance is sample type-dependent.
2. The pellet in step 6 may show light green color when plant leaves are used. If the pellet shows excessive green color the contaminating chloroplast fragment can be removed by resuspend the pellet in 200 µl buffer A by pipetting up and down repeatedly, incubate on ice for 10 min and centrifugate at 4000 X g for 5 min. Save the supernatant (ER fraction) and discard the green pellet.
3. Our in-house research indicates that degree of ER enrichment is sample type-dependent. In most case 1 to 3-fold enrichment can be expected. There is a minimum cross-contamination by Golgi apparatus in the final prep.
4. The enriched ER contains higher concentration of salt. For certain applications such as 2D gel analysis the salt should be removed by a gel filtration column or by dialysis prior to solubilization with proper buffers.

**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. |