Minute™ Rubisco Depletion Kit

Cat. No. RD-046

Description

Plants perceive signals from the environment through leaves. One of the major goals of the scientific community is to dissect the components of signaling cascades by analyzing the leaf proteome. However, the major bottleneck is presence of a high-abundant protein —Rubisco, which accounts for majority of leaf protein ranging from 40-80%. High level rubisco masks the detection and identification of low abundant proteins by LC-MS/MS-based method. A specific IgY conjugated resin is available commercially for depletion of rubisco from extracted total protein but only small amount of protein can be handled and it is very costly. Other methods have been described in the literatures but are usually tedious and time consuming. The Minute™ Rubisco Depletion Kit is designed to overcome these disadvantages by using a specialized filter cartridge coupled with proprietary rubisco depletion buffer. The same, if not better, results can be obtained with a much easier and straighter forward protocol in about 1h. The rubisco-depleted total protein can be used for many downstream applications such as SDS-PAGE, 2DE and LC MS/MS analysis.

Kit Components (20 prep)

1.	Buffer A	15 ml
2.	Buffer B	5 ml
3.	Buffer C	1.0 ml
4.	Filter Cartridge with Collection Tubes	20
5.	Pestle for 1.5 ml microfuge tube	2
6.	Protein Extraction powder	4 g

Additional Materials Required but Not Provided

- Table-top micro centrifuge that can reach 16,000 X g
- 1.5ml Eppendorf tubes
- BCA kit (Pierce) is recommended for protein concentration determination

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

Protocol

Note: Read the protocol carefully before starting. It is recommended to add proteinase inhibitors to aliquot of buffer A prior to use. Pre-chill buffer A on ice. Perform all centrifugation steps at 4°C. Warm buffer B and C to room temperature and mix well prior to use.

1. Place 150-200 mg fresh/frozen leaf in the filter with collection tube. Fold and roll the leaf and insert it into the filter. Add 100 µl buffer A and 80 mg protein extraction powder to the filter**.** Punch the leaf in the filter repeatedly with a 200 µl pipette tip for about 100 times to reduce the volume.
2. Grind tissue with flat end of the pestle with gentle twisting force for about 150-200 times (it takes about 2-3 min. The pestle is reusable. For cleaning, rinse it with water and dry with paper towel.

3. Add 200 µl buffer A to the filter and stir the sample with a 200 µl pipette tip for a few times. Cap the filter and centrifuge at 16,000 X g for 1 min. Discard the filter and resuspend the pellet by pipetting up and down. Transfer all suspension to a fresh 1.5 ml tube
4. Add 80-100 mg protein extraction powder to the tube and grind with a pestle (round end) for 150-200 time with twisting force. Cap the tube and centrifuge at 300 X g for 2 min. Transfer 200 µl supernatant to a fresh 1.5 ml tube (save 50 µl homogenate for total protein control if desired).
5. Centrifuge at 16,000 X g for 30 min. After centrifugation, transfer all supernatant to a fresh 1.5 ml tube and keep on ice (**water soluble fraction**).
6. Add 200 µl buffer A to the pellet (microsomal fraction) slowly, trying not to disturb the pellet. Remove and discard the buffer right away. This step is to wash away residual cytosolic proteins. Resuspend the pellet in 200 µl cold buffer A by pipetting up and down repeatedly followed by addition of 20 µl buffer C to the tube. Mix well and incubate at RT for 5 min. Centrifuge at 16,000 X g for 5 min. The supernatant is **solubilized microsomal protein fraction**. Transfer the supernatant to a fresh 1.5 ml tube and place on ice for later use. Discard the pellet.
7. Add 200 ul buffer B to the tube containing 200 µl water soluble protein from step 5 (supernatant to buffer B ratio = 1:1). Vortex briefly and incubate on ice for 10 min. Centrifuge at 10,000 X g for 5 min. The pellet contains depleted rubisco. Transfer 400 µl supernatant to the tube containing 200 µl solubilized microsomal protein in step 6 above and mix well by vortexing briefly. This is rubisco-depleted total protein extract (600 µl).

*****Since the total protein extract contains high concentration of salts and detergent that need to be removed by TCA precipitation prior to downstream applications such as SDS-PAGE, 2D gel analysis and/or MS spectrometry. We recommend to use high efficiency protein precipitation kit from Invent Biotechnologies, Cat# WA-006. A published modified TCA method can also be used (Niu L. et al. (2018) Modified TCA/acetone precipitation of plant proteins for proteomic analysis. PLoS ONE 13(12): e0202238. https://doi.org/10.1371/journal.pone.0202238).***

Tech Note:

1. The amount of starting material is suitable for most leaf samples. Don't use more than 200 mg/sample. More is not necessarily better.
2. After TCA precipitation the final protein yield is in the range of 100-200 µg/sample for most leaf tissues.
3. This protocol combines solubilized microsomal fraction with water soluble fraction to obtain rubisco-depleted total protein. The microsomal fraction and water-soluble protein fraction can be precipitated separately if desired. However, 20 µl buffer C should be mixed with 400 µl supernatant from step 7 in a fresh 1.5 ml tube prior to TCA precipitation.
4. If desired, the water-soluble protein fraction (from step 5) can be used directly for applications such as immunoprecipitation without rubisco depletion and TCA precipitation.