Minute™ Water-soluble and Water-Insoluble Protein Fractionation Kit

Catalog Number WS-056

Description

Researchers studying protein localization and trafficking now have a convenient tool: the Minute™ Water-soluble and Water-Insoluble Protein Fractionation Kit. This unique product allows for the rapid and efficient separation of these two key fractions from cells/tissues, microorganisms and plant tissues, providing enriched and easily detectable protein samples for downstream applications like western blotting, ELISA and immunoprecipitation. Water soluble fraction contains mainly cytosolic proteins and water-insoluble fraction contains mainly insoluble membranous structures such as plasma membrane, nuclear membrane and organelles. The kit offers researchers a novel and simple approach for understanding protein function and movement within the cellular landscape. If a protein target is known to be localized in water-soluble or water-insoluble fraction, the kit will facilitate detection, isolation and purification of the target protein by enrich corresponding fractions.

Applications

The kit is designed to rapidly separate cell/tissues protein into water-soluble and water-insoluble fractions for applications such as SDS-PAGE, immunoblotting, ELISA, IP, protein localization, gel mobility shift assays, and other applications.

Kit Components (20 preps)

1.	Buffer A	15 ml
2.	Buffer B	25 ml
3.	Buffer C	1 ml
4.	Buffer D	1 ml
5.	Buffer N	1 ml
6.	Protein extraction powder	2.0 g
7.	Pestle for 1.5 ml microfuge tube	2
8.	1.5 ml microfuge tube	20

Storage: Store the kit at room temperature.

Additional Materials Required

Table-Top Microcentrifuge. 1 X PBS

Important Product information

A. Read the protocol carefully before start. The buffers of this kit do not contain protease inhibitors. For best results, addition of protease inhibitors (such as cOmplete from Roche) to aliquot of the

cell lysis buffer is recommended. The BCA assay (Pierce) is recommended for determining protein concentration. For protein phosphorylation studies, **phosphatase inhibitors** (such as PhosStop from Roche) must be added to the aliquot of cell lysis buffer prior to use. Unless specified, centrifugation and incubation steps can should be done at 4°C. The pestle is reusable. For cleaning, wash it with ddH2O and dry with paper towel or wipe it with 75% alcohol.

B. Chill buffer A and B on ice prior to use.

Protocol

Cell/Tissue Homogenization

A. For cultured cells:

1. Harvest cells from culture containers by scraping (adherent cells) or by centrifugation (cells in suspension). Cell# can be ranging from 5 to 50 million.
2. Wash cells once with cold 1 X PBS. Resuspend cell pellet in 1 ml PBS and transfer to a 1.5 ml microfuge tube provided.
3. Centrifuge at 600 X g for 5 min and remove supernatant completely. Add 80-100 mg protein extraction powder to the bottom of the tube followed by addition of 50 µl buffer A.
4. Homogenize the cells with the pestle provided by twisting back and forth for about 200-300 times. Add 100 to 500 µl buffer A (see tech notes below) to the tube and continue to homogenize a few more times.
5. Cap the tube and vortex vigorously for 10-20 seconds. Centrifuge at 600 X g at 4°C for 3 min. Transfer all supernatant to a fresh microfuge tube and go to step 1 of cell fractionation below.

B. For animal tissues:

1. Add 10-40 mg animal tissue to a 1.5 ml microfuge tube provided followed by addition of 80-100 mg protein extraction powder and 50 µl buffer A to the bottom of the tube.
2. Homogenize the tissue as in A above. Add 100-500 µl buffer A to the tube. Cap the tube and vortex vigorously for 10-20 seconds and process as described above and go to step 1 of cell fractionation below.
3. For hard tissues such as skeletal muscle, cardio muscle and skin tissue, mince them with a sharp blade into tissue slurry prior to homogenization to increase protein extraction efficiency.

C. For microorganisms (bacteria/yeast/others):

1. Harvest cultured microorganism by centrifugation. Wash cell pellet with 1 X PBS once. Resuspend the pellet in 1 ml PBS and transfer cell suspension to a 1.5 ml tube provided.
2. Centrifuge at 5,000 X g at 4°C for 5 min to pellet the cells. The wet volume of pellet should be about 80-100 µl for bacteria and 100-150 µl for yeast.
3. Remove supernatant completely and add 50 µl buffer A and 80-100 mg protein extraction powder to bottom of the tube. Homogenize the cells pellet with the pestle by twisting back and forth for about 300-400 times. This will take about 2-3 min.
4. Add 200 to 400 µl buffer A (see tech notes below) to the tube and continue to homogenize a few times. Cap the tube and vortex vigorously for 10-20 seconds. Centrifuge at 2,000 X g for 5 min. Transfer all supernatant to a fresh microfuge tube. Go to step 1 of cell fractionation below.

D. For plant tissues:

1. For leaves, fold or roll 50-300 mg of fresh tissue and insert into a 1.5 ml microfuge tube. Punch the leaf repeatedly with a 1 ml pipette tip to reduce the volume. For seeds (fresh/frozen/soaked), soft stems, roots, etc., cut them into smaller pieces using a sharp blade. Place the tissue into the tube.
2. Add 80-100 mg protein extraction powder and 100 µl buffer A into the tube.
3. Homogenize the sample with the pestle by twisting back and forth for about 300-400 times. This will take about 2-3 min.
4. Add 100 to 400 µl buffer A to the tube and continue to homogenize a few times. Cap the tube and vortex vigorously for 10-20 seconds. Centrifuge at 700 X g for 5 min. Transfer all supernatant to a fresh microfuge tube. Go to step 1 of cell fractionation below.

Cell Fractionations

1. Centrifuge the supernatant at 16,000 X g for 30 min to 1h at 4°C.
2. Transfer the supernatant into a fresh 1.5 ml microfuge tube (**this is water-soluble fraction**).
3. Resuspend the pellet (**water-insoluble fraction**) in 0.5 ml buffer B by pipetting up and down for 20-30 times and add 10 µl buffer C to the tube and mix well by vortexing briefly.
4. Centrifuge at 16,000 X g for 10-20 min. This is to wash water-insoluble fraction to reduce contamination by water-soluble proteins. Repeat step 3 and 4 one more time. The pellet is water-insoluble fraction.

Solubilization of water-insoluble Fraction

1. Resuspend water-insoluble fraction in 50-200 µl buffer B (depending upon pellet size).
2. Depending upon downstream applications, the water-insoluble fraction can be dissolved by mix the resuspended pellet with 1/10th volume of buffer D or buffer N (for example: mix 100 µl of resuspended pellet with 10 µl of buffer D or N). After solubilization, protein concentration can be determined by BCA assay.
3. Buffer D contains denaturing detergent and buffer N contains non-denaturing detergent. Denatured proteins can be used for SDS-PAGE or Western blotting. Non-denatured proteins can be used for ELISA or immunoprecipitation or other experiments.

Tech notes

1. After homogenization, the amount of buffer A added can be ranging from 100-500 µl depending upon the amount of starting material. Generally, the protein concentration of water-insoluble fraction is 0.5-1 mg/ml and that of water-soluble fraction is 2-4 mg/ml.
2. Increase homogenization time usually can increase protein yield especially for microorganism samples.
3. For better protein solubilization, the amount of buffer D or N can be doubled. For example: 20 µl buffer D or N can be mixed with 100 µl water-insoluble suspension.
4. The use of buffer C is to increase the washing stringency. Higher stringency washing buffer may strip some protein non-specifically associated with water-insoluble fraction. For certain

applications such as immunoprecipitation or co-immunoprecipitation, buffer C may be omitted.

5. To increase the washing stringency 20 µl buffer C can be mixed with 500 µl buffer B in step 3 of cell fractionations.
6. For samples of microorganisms and plants, the water-soluble fraction may contain some floating cell wall materials after 16,000 X g spin. The water-soluble fraction can be spin again at 16,000 X g for 5 min and insert a pipette tip under the floating material to obtain clear water-soluble fraction.