Minute™ Adipose Tissue Fractionation Kit

Catalog number: AF-023

Description

In average, total cellular proteins account for less than 2% of adipose tissues. Fractionation of adipose tissue is technically very challenging due to its high lipid and low protein contents. Adipose tissue especially white adipose tissue (WAT) has been recognized as an important endocrine and inflammation organ in addition to its energy storage function. Fractionation and analysis of proteins from adipose tissues are critical for understanding many physiological/pathological conditions. The water-oil emulsion present in biological sample is notoriously difficult to separate. We have developed a novel technology to deal with this issue. A porous filter with unique surface property and pre-defined pore size coupling with a specially formulated detergent-free fractionation buffer is the key to fractionate adipose tissue into two fractions: water-soluble protein fraction containing mainly cytosolic proteins and water insoluble fraction containing mainly plasma membrane, and organelles such as mitochondria. The buffers used in this kit are free of primary amine, detergent and reducing agents. Isolated proteins are compatible with all downstream applications including TMT labeling, enzyme digestion, MS analysis and other applications.

Application

MS analysis, 2D gel analysis, ELISA, SDS-PAGE, immunoblottings, immunoprecipitation, enzyme activity assays, and other applications.

Kit components

1. 15 ml buffer A
2. 15 ml buffer B
3. 1.5 ml microfuge tube (20)
4. Pestles for 1.5 ml tube (2)
5. 20 protein extraction filter cartridges with collection tubes
6. Protein Extraction Powder (2 g)

Storage: Store the kit at 4C

Additional Materials Required: Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 rpm.

Important Product Information

The use of protease inhibitors is optional. However if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time,

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addition of protease inhibitor to buffer A is recommended. For determination of protein concentration BCA assay (Pierce) is recommended. For protein phosphorylation studies, cocktails of phosphatase inhibitors need to be added to buffer A prior to use. **Perform all centrifugation steps in a cold room or in a refrigerated microcentrifuge.**

Protocol For

Adipose Tissues (WAT or BAT) Fractionation

1. Pre-chill buffers A and a filter cartridge in collection tube on ice.
2. Weight out 200-300 mg fresh or frozen adipose tissue, place it between a few layers of paper towel and squeeze with thumb and index finger to remove excessive fat from the tissue. Place the tissue in the bottom of a 1.5 ml microfuge tube provided using a forceps (**don't use other 1.5 ml tube from your Lab because it may not fit the pestle provided**). Weigh out 100 mg protein extraction powder and add to the tube on top of the tissue. Add 50 µl buffer A to the tube.
3. Grind the tissue with a pestle with twisting force for about 1-2 min to reduce the tissue to slurry. Add 500 µl buffer A to the filter and continue to grind for another 1 min. The pestle is reusable. For cleaning wipe it with alcohol and air dry.
4. Cap the tube and centrifuge at 2,000 rpm for 1 min. Go under the aggregated fat on top and transfer 400 µl supernatant to a filter cartridge with collection tube (it does not matter if some fat aggregate is carried over).
5. Incubate the filter cartridge with cap open on ice for 10 min. Cover the ice bucket with a lid or a piece of paper. After incubation immediately cap the filter cartridge and centrifuge at 2000 rpm for 1 min.
6. Discard the filter cartridge and centrifuge at 14,000 to 16,000 rpm for 20 min. After centrifugation **pour** the supernatant (water-soluble fraction) into a fresh 1.5 ml tube from your Lab (**the 1.5 ml tube provided is for use in step 2 only**) and incubate on ice (in many cases there is a thin layer of aggregated fat on top of the supernatant, it will be removed in step 7). If there is aggregated fat attaching to the wall of the tube wipe it out with a small piece of paper towel.
7. Resuspend the pellet in 400 µl buffer B by pippetting up and down. Centrifuge the tube with resuspended buffer B and the tube containing supernatant from step 6 at 14,000-16,000 rpm for 15–20 min. Remove buffer B completely and save the pellet (this is the water-insoluble fraction containing mainly plasma membrane and organelles). You can dissolve the pellet in 50-60 µl detergent containing buffer of your choice. The protein yield is typically about 30-60 µg/sample depending upon the tissue types. If more proteins are needed simply run multiple samples at the same time and combine the pellets in the final step. To obtain a oil-free water soluble fraction simply insert a pipette tip to the bottom of the tube containing supernatant from step 6 and transfer the aqueous portion to a new tube. The protein concentration of the water soluble fraction is typically 2-3 mg/ml.

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Protocol for Cultured Adipocyte Fractionation

1. Harvest 50-100 million cultured adipocyte by low speed centrifugation. Resuspend the cells in a 1.5 ml tube with 1ml cold PBS that contains phosphatase inhibitor or protease cocktails as recommended above. Add 100 mg protein extraction powder to the tube.
2. Centrifuge in a microfuge at 3000 rpm for 3 min. Remove supernatant completely. Grind the cells with pestle with twisting force for about 1-2 min to homogenize the cells. Add 500 µl buffer A to the tube and continue to homogenate for another 30 seconds.
3. Centrifuge at 2000 rpm for 1 min and transfer 400 µl the supernatant to a pre-chilled filter cartridge with collection tube. Follow step 5-7 above.

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