Minute™ Total Protein Extraction Kit for Adipose Tissues/Cultured Adipocytes

Catalog number: AT-022

Description

Adipose tissue, especially white adipose tissue (WAT), has been recognized as an important endocrine and inflammation organ in addition to its energy storage function. Isolation and analysis of proteins from adipose tissues are increasingly critical for understanding many physiological/pathological conditions. However, isolation and analysis of WAT and brown adipose tissue (BAT) are technically very challenging due to their high lipid and low protein contents. The water-oil emulsion present in biological sample is notoriously difficult to separate. We have developed a novel technology to address this issue. A porous filter with unique surface property and pre-defined pore size and thickness coupling with a specially formulated detergent-free extraction buffer is employed to rapidly and effective separate water-oil emulsion derived from adipose tissue homogenate. The extraction buffer has lower freezing point than that of oil in adipose tissues and the aqueous phase can be quickly separated from oil phase by passing the tissue homogenate through the filter. The total proteins isolated are un-biased representation of cellular proteins in the tissue. The extracted proteins concentration is very high (2-3 mg/ml) as compared to other methods.

Application

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

Kit components

1. 15 ml buffer A (extraction buffer)
2. 1.5 ml buffer B (10 X denaturing buffer)
3. 1.5 ml buffer C (10 X non-denaturing buffer)
4. 1.5 ml microfuge tube (20)
5. Pestles for 1.5 ml tube (2)
6. 20 protein extraction filter cartridges with collection tubes
7. Protein Extraction Powder (2 g)

Shipping: This kit is shipped at ambient temperature
Storage: Store Buffer A at 4°C and rest of the kit at RT

Additional Materials Required

Table-Top Microcentrifuge

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, addition of protease

inhibitor to buffer A is recommended. For determination of protein concentration BCA assay (Pierce) is recommended. Bradford assay is not compatible with the kit. For protein phosphorylation studies, cocktails of phosphatase inhibitors need to be added to buffer A prior to use.

If precipitate is found in buffer B in the winter time, incubate at >37^{0}C until the precipitate is completely dissolved.

Protein Extraction Protocols

Protein Extraction from Adipose Tissues (WAT or BAT)

1. Pre-chill buffers A and a filter cartridge in collection tube on ice.
2. Weight out 50-80 mg fresh or frozen adipose tissue, place it between a few layers of paper towel and squeeze with thumb and index fingers to remove a portion of oil from the tissue. Use forceps to place the tissue in the bottom of a 1.5 ml microfuge tube provided (**don't use other 1.5 ml tube from your Lab because it may not fit the pestle provided**). Weigh out 80-100 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 200-300 µl buffer A to the tube. and continue to grind for another 30 seconds. If smaller amount of starting tissue (20-40 mg) is used add 100-150 µl buffer A to the tube.
4. Cap the tube and centrifuge at 350 X g for 1 min. Transfer supernatant to a filter cartridge with collection tube (it does not matter if some fat aggregate is carried over. The pestle is reusable. For cleaning, wipe it with alcohol and air dry).
5. Incubate the filter cartridge with cap open at -20C for 15-20 min. Check the temperature of your refrigerator to make sure that the temperature is around -20C. Otherwise refer to trouble shooting below.
6. After incubation immediately centrifuge at 350 X g for 1-2 min with cap open. The flow through contains total proteins from adipose tissue. Extracted proteins appear slightly clouded due to the presence of water insoluble cellular components. It can be diluted and directly use in ELISA for detection of water soluble proteins. It can also be resuspended in buffer B or buffer C to dissolve water insoluble proteins for downstream applications:

   A. Add 1/10 of Buffer B to the extracted protein solution resulting in a denatured protein solution (ideal for SDS-PAGE, Westerns and other applications) or
   B. Add 1/10 of Buffer C to the extracted protein solution resulting in a non-denatured protein solution (ideal for IP, ELISA and other applications) or
   C. Dissolve in 2 X 2D gel sample buffer for 2D gel analysis.

Note: Buffer A contains a component that may interfere with mass spec analysis. If extracted proteins are used for MS analysis, dialysis against compatible buffer first. Another option is to precipitate proteins using standard TCA protein precipitation protocol.

Protein Extraction from Cultured Adipocytes

1. Harvest 30-50 million cultured adipocytes by low speed centrifugation. Resuspend the cells in a 1.5 ml tube with 1 ml cold PBS that contains phosphatase inhibitor or protease cocktails as recommended above. Add 80 mg protein extraction powder to the tube.

2. Centrifuge at 500 X g for 3-5 min. Remove supernatant completely. Grind the cells with pestle with twisting force for about 1-2 min to homogenize the cells. Add 200-300 µl buffer A to the tube and continue to homogenate for another 30 seconds.
3. Centrifuge at 350 X g for 1 min and transfer the supernatant to a pre-chilled filter cartridge with collection tube. Follow step 5-6 above.

Troubleshooting:

This protocol is simple and straight forward however incubation time of step 5 is critical for clear separation of aqueous phase from oil phase in the tissue homogenate. Because there are variations in the actual temperature of the refrigerator in a particular lab, we recommend performing a simple test to determine the optimal incubation time:

Add 0.5 ml ddH2O to a **1.5 ml microfuge tube used in your lab** and incubate in your refrigerator with cap open. Determine minimum time required to freezing the water completely. This is the optimal incubation time for step 5. You can also perform this test in a -70-80C freezer. The use of -70-80C freezer will significantly reduce the incubation time in step 5.