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 essential endocrine and inflammation organ in addition to its energy storage function. Analyses of proteins from adipose tissues are increasingly critical for understanding many physiological/pathological conditions. However, isolation of WAT and brown adipose tissue (BAT) is technically challenging due to their high lipid and low protein contents. The water-oil emulsion present in a 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 effectively separate water-oil emulsion derived from adipose tissue homogenate. The extraction buffer has a lower freezing point than oil in adipose tissues, and the aqueous phase can be quickly separated from the oil phase by passing the tissue homogenate through the filter. The total proteins isolated are the unbiased representation of cellular proteins in the tissue. The extracted proteins concentration is very high (2-3 mg/ml) compared to traditional 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 the downstream application takes significant amounts of time or the protein extract will be stored for a more extended time, the addition of protease inhibitor(s) to buffer A is recommended. For the determination of protein concentration, A BCA assay

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

If precipitate is found in buffer B at a lower temperature, incubate a 37°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 of fresh or frozen adipose tissue, place it between a few layers of paper towel and squeeze with your thumb and index fingers to remove a portion of the 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 tubes because they 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 the twisting force for about 1-2 min to reduce the tissue to the slurry (The pestles are reusable. For cleaning, wipe with alcohol and air dry). Add 200-300 µl buffer A to the tube and continue to grind for another 30 seconds. If a smaller amount of starting tissue (20-40 mg) is used, add 100-150 µl buffer A to the tube.

   Note: The buffer A can be increased to 500 µl if desired, but the protein concentration may be reduced.

4. Cap the tube and centrifuge at 350 X g for 1 min. Transfer the supernatant to a filter cartridge with a collection tube (it does not matter if some fat aggregates are carried over).
5. Incubate the filter cartridge with the cap open at -20C for 15-20 min. Check the temperature of the freezer to make sure that the temperature is around -20°C. Otherwise, refer to the tech note below.
6. After incubation, immediately centrifuge at 350 X g for 1-2 min with the cap open. The flow-through contains total proteins from adipose tissue, which may appear to be slightly cloudy due to the water-insoluble cellular components. It can be diluted and used directly in ELISA for detection of the water-soluble proteins.

   Note: The centrifugal force can be increased to 600 X g for 2 min if buffer retention is found in the filter.

   The sample can also be resuspended in buffer B or C so the water-insoluble proteins are dissolved 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. Standard TCA precipitation or dialysis against a compatible buffer is required.

Protein Extraction from Cultured Adipocytes

1. Harvest 30-50 million cultured adipocytes by low-speed centrifugation (1000 X g 5 min) using 1.5 ml Eppendorf tubes and wash once with PBS. Remove the medium or PBS with a syringe if the adipocytes float on the top after centrifugation.
2. Add 80 mg protein extraction powder (add phosphatase inhibitor or protease inhibitor cocktail as needed), and grind using a pestle with the twisting force for 1-2 min. Add 200-300 µl buffer A 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 a collection tube. Follow steps 5-6 for adipose tissues above.

Tech Note:

1. For BCA assay (Pierce), we recommend using phosphate-buffered saline (PBS) as a diluent for standard curve construction. Dilute the extracted protein sample and the buffer A at a 1:4 ratio with PBS, and use diluted buffer A as a negative control (zero the spectrophotometer) for optical density measurement.

2. This protocol is simple and straightforward; however, the incubation time of step 5 is critical for clear separation of the aqueous phase from the oil phase in the tissue homogenate. Due to the variations in the actual temperature of the freezer 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 Eppendorf tube and incubate in the freezer with the cap open. Determine the minimum time required for the water to be frozen completely. This is the optimal incubation time for step 5. A -70 or 80°C freezer may also be used to significantly reduce the incubation time.

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