Total protein extraction from adipose tissue and cells

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ABSTRACT

Adipose tissue, especially white adipose tissue (WAT), has been shown to be associated with endocrine and organ inflammation in addition to energy storage. Extraction and analysis of proteins from adipose tissue is increasingly important for understanding many physiological/pathological conditions. However, due to the high fat and low protein content in white adipose tissue (WAT) and brown adipose tissue (BAT), it is technically challenging.

It is well known that water-oil emulsions in biological samples are the most difficult to separate, and the pore-sized centrifugal column with unique surface properties and the optimized surfactant-free buffer system can quickly and efficiently emulsify water oil from adipose tissue homogenate. The extraction buffer is lower than the oil freezing point in the adipose tissue, and the adipose tissue homogenate can quickly separate the aqueous phase and the oil phase through the centrifugal column, and the total protein in the tissue is not lost. Protein yields can reach 2-3 mg/ml, which is much higher than other methods.

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 Place buffer A, centrifuge column and receiver tube sleeve on ice for pre-cooling.

2 Weigh 50-80 mg of fresh or frozen adipose tissue, place it on several layers of paper towels, squeeze with your thumb and forefinger, and remove a portion of the oil from the tissue. Place the tissue into the 1.5 ml centrifuge tube supplied with the kit with tweezers and weigh 100 mg of protein extract powder into the sample. Add 50 ul of buffer A.

3 Twist the ground tissue sample with a grinding rod for 1-2 minutes. Add 200-300 ul Buffer A and continue grind the sample for 30 seconds. If the initial amount of tissue is small (20-40 mg), 100-150 ul of buffer A is required.

4 Cap the lid and centrifuge at 2000 rpm for 1 minute. Transfer the supernatant to a centrifuge column placed in the receiver tube.

5 Incubate at - 20 ° C for 15-20 minutes. (Please make sure the refrigerator temperature is around - 20 °C.)

6 Centrifuge at 2,000 rpm for 1-2 minutes after incubation. Discard the centrifuge tube and obtain the total protein in the adipose tissue. The extracted protein contains a small amount of insoluble matter as a water-insoluble cellular component. It can be diluted and used directly in ELISA to detect water soluble proteins. It is also possible to resuspend the water-insoluble protein in buffer B or C for downstream applications:

7 Add 1/10 buffer B to the extracted protein solution to become a denatured protein solution (for SDS-PAGE, Western and other applications)

8 Add 1/10 buffer C to the extracted protein solution to become a non-denaturing protein solution (for IP, ELISA and other applications)

9 Dissolve in 2X 2D gel sample buffer for 2D analysis.

Extracting proteins from fat cells

10 Collect 50-100 million fat cells by low speed centrifugation. Resuspend the cells by adding 1 ml of pre-cooled PBS (phosphorase inhibitor or protease inhibitor according to the above recommendations) to a 1.5 ml centrifuge tube. Add 100 mg protein extract powder.

11 Centrifuge at 3000 rpm for 3 minutes and discard the supernatant. Twist the grinding with a lapping rod for 1-2 minutes to homogenize the cells. Add 200-300 ul Buffer A and continue grind for 30 seconds.

12 Centrifuge at 2000 rpm for 1 minute, then transfer the supernatant to the pre-cooled centrifuge column receiver tube cannula.