Minute™ Single Nuclei and Cytosol Isolation Kit for Adipose Tissues/Cultured Adipocytes

Cat. No. AN-029

Description

Adipocytes are the major energy storage sites in the body, and they also have critical endocrine functions. Therefore, understanding the development and function of adipocytes is essential to understanding metabolic homeostasis under physiological and pathological conditions. Fractionation of cellular components into a single nucleus and cytosolic fraction is a common lab practice. However, when it comes to adipose tissue and adipocytes, separating these two fractions is quite challenging due to the low protein content and the presence of abundant lipid droplets. Methods developed by different labs are tedious and unpractical, requiring up to 50 grams of starting materials. This kit provides a quick and simple protocol for obtaining high-quality single nuclei and cytosol fraction from adipose tissue and adipocytes. Only milligrams of starting material are required, making it possible to isolate nuclei and extract proteins from biopsies and limited tissues. The intact nuclei isolated can be used in many applications such as scRNA-seq, ATAC-seq, SDS-PAGE, immunoblotting, ELISA, IP, protein localization, gel mobility shift assays, 2-D gels, and other applications.

Kit Components

N/C Buffer	15 ml
1.5 ml Tube	20
Pestle for 1.5 ml tube	2
Filter Cartridge	20
Collection Tube	20

Material required but not supplied: 1 X PBS (calcium-free) with 5% BSA
Shipping and Storage: Shipped at ambient temperature and stored at 4°C.

Important Information:

1. Read the entire procedure carefully. Put filter cartridges in collection tubes and sit on ice before use.
2. Make sure the temperature of the freezer is about -20°C.
3. Add p**hosphatase inhibitors** (such as PhosStop from Roche) to the N/C buffer prior to use for phosphorylation studies. **Protease inhibitors** may also be needed (optional) if the cytosolic friction is to be used for downstream experiments.
4. It is recommended to use BCA Protein Assay Kit to determine the protein concentration (Pierce, Cat #:23227).
5. RNAse inhibitors should be added to an aliquot of N/C buffer if isolated nuclei are intended for RNA-related experiments such as scRNA-seq.

Protocol

Unless specified, the following procedures are performed at room temperature. Fresh or frozen tissues can be used. For frozen tissue, thaw out at 37°C.

For Adipose Tissues

1. Weight out 150-160 mg of fresh/frozen tissue (more than 160 mg of tissue is not recommended) and cut it into smaller pieces (about 2-3 x 2-3 mm). Transfer the tissues to the bottom of a 1.5 ml tube provided and add 200 µl of N/C buffer.
2. Homogenize with the pestle provided by grinding and pushing against the bottom and sidewall gently for 2-3 min. Add 500 µl of N/C buffer and continue to grind 20-30 times.
   (The pestle is reusable. Wipeout with a paper towel and clean with 70% alcohol.)
3. Place a filter cartridge in a collection tube, and pour the homogenate into the filter cartridge (it's ok if some tissue debris is carried over).
4. Incubate the filter cartridge with the cap open in a -20°C freezer for 10-15 min. After incubation, cap the tube and immediately centrifuge at 13,000 x g for 15-20 seconds using a tabletop centrifuge.
5. Discard the filter, and resuspend the pellet by pipetting up and down gently using a 1 ml tip (try to avoid fate cake on the wall as much as possible) for about 10-20 times. Transfer all homogenate to a 1.5 ml microfuge tube and centrifuge at 1200 X g for 5 min.
6. Pour out all supernatant (this is the cytosolic fraction, save it if desired), and use a cotton swab to wipeout any fat stick on the wall with the tube positioned upside-down. Do not touch the nuclear pellet at the bottom.
7. Resuspend the pellet (in most cases, it is invisible) in 30-50 µl PBS with 5% BSA by gently pipetting up and down 10-20 times. The isolated nuclei can be examined under a microscope with trypan blue or DNA stain (requiring a fluorescence microscope). Typically, 50,000-100,000 well-separated nuclei can be obtained, which can be used for FACS, snRNA-seq, scATAC-seq, scDNA-seq, and extraction of total nuclear proteins/DNAs/RNAs. The protein concentration of the cytosolic fraction is about 0.5-1 mg/ml.

For Cultured Adipocytes

1. Harvest cultured adipocytes (10-20 million) by low-speed centrifugation (1000X g for 5 min) using 1.5 ml Eppendorf tubes and wash twice with cold PBS.
   Note: Remove the medium or PBS with a syringe if the adipocytes float on the top after centrifugation
2. Add 0.7 ml N/C buffer to the pellet and resuspend by pipetting up and down 10-15 times, and incubate at -20oC for 10 min.
3. Follow the steps from 3-7 above

Tech Note:

1. The isolated nuclei are pretty clean and well separated. If higher purity is desired, they can be further sorted by flow cytometry.
2. If a cotton swab is not available, the fate cake can be wiped out by using a pair of forceps with a small piece of paper towel.
3. The cytosolic fraction can be obtained by centrifuging supernatant mix in step 6 at 12.000 X g for 10 min at 4°C. Transfer the clear cytosolic fraction to a new tube.
4. For RNA-seq applications, it is recommended to enzymatically digest the fate tissue to isolate adipocytes first. This will give much higher number of nuclei (see reference below).

Rajbhandari, P., Arneson, D., Feng, A. C., Ahn, I. S., Diamante, G., Zaghari, N., ... & Smale, S. T. (2019). Single Cell Analysis Reveals Immune Cell-Adipocyte Crosstalk Regulating the Transcription of Thermogenic Adipocytes. *eLife 2019;8:e49501*