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Minute^{TM} 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 single nucleus and cytosolic fraction is a common practice in the lab. However, when it comes to adipocytes, separation of these two fractions is much more difficult because of the high concentration of lipid droplet and low protein content of adipocytes. Methods reported in the literature are tedious and time-consuming and as much as 50 grams of tissue are required. This kit provides a very rapid and simple method for obtaining high purity single nuclei from adipose tissues, and most important of all, only milligram amounts of tissues are required making it possible to isolate nuclei and cytosol from small animals and biopsy samples. The intact nuclei isolated have many applications such as single nucleus RNA sequencing, 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 store at 4°C.

Important Information:

1. Read the entire procedures carefully. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. Make sure your freezer is about -20°C.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to N/C buffer prior to use. The use of protease inhibitor cocktails is optional. If you are interested in use of cytosolic protein for downstream experiment, protease inhibitors should be added to N/C buffer prior to use.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

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5. If isolated nuclei are intended for RNA related work such as single nucleus RNA sequencing, RNAse inhibitors should be added to aliquot of N/C buffer.

Protocol

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

For Adipose Tissues

1. Weight out 150-160 mg fresh/frozen tissue (more than 160 mg tissue is not recommended) and cut it into smaller pieces (about 2-3 X 2-3 mm). Place the tissues in the bottom of 1.5 ml tube provided and add 200 µl N/C buffer to the tube.
2. Homogenize the tissue with a pestle provided by grinding and pushing against the bottom and side wall of the tube gently for two to three min. After homogenization, add 500 µl N/C buffer to the tube and continue to grind for 20-30 times. The pestle is reusable (wipe out the fat cake attached to the pestle with a piece of paper towel and clean it with 70% alcohol).
3. Place a filter cartridge in a collection tube and pour all homogenate from above tube to the filter (it's ok if some fat tissue is carried over).
4. Incubate the filter cartridge with cap open at -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 table top centrifuge.
5. Discard the filter, resuspend the pellet by pipetting up and down gently using a 1 ml tip (try to avoid fate cake attaches to the wall of the tube but it's ok if some fate cake is resuspended) for about 10-20 times. Transfer all homogenate to a 1.5 ml microfuge tube. Cap the tube and centrifuge at 1200 X g for 5 min.
6. After centrifugation pour out all supernatant (this is cytosolic fraction, save it if desired). **Important:** After pour out the supernatant, keep the tube opening in downward position and use a cotton swab to wipe out any fate that adheres to the wall of the tube. Try not to touch the nuclear pellet at the bottom of the tube.
7. Turn the tube opening upward and resupend the pellet (in most cases it is invisible) in 30-50 ul PBS with 5% BSA by gently pipetting up and down for 10-20 times. The yield of isolated nuclei can be determined by a microscope using trypan blue staining. DNA stain can also be used if a fluorescence microscope is available. Typically, 50,000-100,000 nuclei can be obtained/sample. The protein concentration in the cytosolic fraction is about 0.5-1 mg/ml. Isolated nuclei can be used for extraction of protein, DNA and RNA.

For Cultured Adipocytes

1. Harvest cultured adipocytes (10-20 million) by low-speed centrifugation (500-700 X g for 5 min) and wash the cells twice with cold PBS. Remove PBS completely.
2. Add 0.7 ml N/C buffer to the pellet and resuspend by pipetting up and down for 10-15 times. Incubate at -20°Cfor for 10 min.
3. Follow the ^{steps} from 3-7 above)

Tech Note:

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1. The nuclei isolated with above protocol 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 isolated 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 then use the kit to isolate nuclei. This will give much higher number of nuclei (see reference below).

Reference:

1. 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. *bioRxiv*, 669853

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