Minute™ Nuclei and Cytosol Isolation Kit for Adipose Tissues

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 are essential to understanding metabolic homeostasis under physiological and pathological conditions. Fractionation of cellular components into nucleus and cytosolic fraction is a common practice in the lab. However, when it comes to adipocytes, separation of these two fractions are much more difficult because of 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 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 animal and biopsy samples.

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

Shipping and Storage: Shipped and stored at ambient temperature.

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^{o}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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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. The rpm specified below is based on 5415C table top centrifuge (Eppendorf).

1. Weight out 120-150 mg fresh/frozen tissue (more than 160 mg tissue is not recommended) and cut into smaller pieces (about 2-3 X 2-3 mm). Place the tissues in the bottom of 1.5 ml tube provided and add 600 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, you should see significant amount of fat cake attached to the side wall of the tube and the head of pestle. 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 the liquid 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 20 min (make sure the temperature of the freezer is around -20°C). After incubation, immediately place the tube with cap open in a table top microcentrifuge and centrifuge at 2000 rpm (500 x g) for 2 min (if there is liquid retention in the filter, increase centrifugation to 1000 X g for 1 min).
5. Discard the filter, cap the tube and vortex briefly. Centrifuge the tube at 4000 rpm (1000 X g) for 4 min. Pay attention to the position of your tube and know where the nuclei pellet should be located because the isolated nuclei are transparent and, in most cases, invisible.
6. Remove and/or save the supernatant (this is cytosolic fraction). Resuspend the nuclei pellet in 30 µl PBS or other buffer of your choice. 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 intact 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.

Trouble shooting

This protocol is simple and straight forward however incubation time of step 4 is critical for clear separation of aqueous phase from oil phase in the tissue homogenate. Due to variations in the actual temperature of the refrigerator in a particular lab, if significant lipid contamination is encountered, we recommend to perform following 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 4.

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