Minute™ Cytosolic and Nuclear Isolation Kit for Metabolite Analysis

Catalog number: MT-059

Description

This detergent-free cytosolic and nuclear isolation kit is designed to generate samples for metabolite analysis from cultured cells and tissues. Intact nuclei can be isolated in less than 40 minutes without the use of Dounce homogenizers or detergents. Traditional nuclear isolation methods typically rely on non-ionic detergents, which can interfere with mass spectrometry and may cause leakage of nuclear metabolites.

In this method, cells or tissues are first sensitized with Buffer A and then passed through a proprietary filter in a zigzag path under high-speed centrifugal force. Cells rupture as they pass through the filter, while intact nuclei are released into the flow-through. The nuclei are subsequently separated from the cytosolic fraction by centrifugation, and nuclear metabolites are extracted using methanol or other suitable solvents.

Kit components

1.	Buffer A	15 ml
2.	Buffer B	20 ml
3.	Buffer C	25 ml
4.	Filter cartridges	20
5.	Collection tubes	20
6.	Pestle for 1.5 ml tube	(2)
7.	Tissue dissociation beads	(2.0g)

Shipping: This kit is shipped at ambient temperature**.**
Storage: Store the kit at 4ºC**.**

Additional Materials Required but not provided

1. Table-Top Microcentrifuge with >14,000 rpm (16,000 × g)
2. Methanol (LC-MS grade)

Important information

The purity and integrity of the isolated nuclei depend on the quality and type of the cells or tissues, and yields may vary accordingly.

Protocol for Mammalian Tissues

(Pre-chill buffers on ice. Perform all centrifugation steps at 4°C)

1. Add 20–30 mg of fresh or frozen soft tissue to the filter cartridge. For frozen tissues, thaw completely on ice before use. For muscle tissues, place the tissue on a clean glass or plastic plate and mince thoroughly with a sharp blade to generate a tissue slurry or paste, then transfer it to the filter cartridge. If the yield is low, the addition of the included tissue dissociation beads may improve recovery (see Tech Notes below).
2. Add 200 µL of cold Buffer A to the filter cartridge. Grind the tissue for approximately 1–2 minutes using the flat end of the provided pestle (the pestle is reusable and can be cleaned by rinsing with water). For muscle tissue, double the grinding time.
3. Add 300 µL of cold Buffer A to the same filter cartridge and incubate on ice for 5–10 minutes with the cap open. For muscle tissue, extend the incubation to 20–30 minutes. After incubation, cap the tube and gently invert several times to mix.
4. Centrifuge in a tabletop microfuge at 16,000 × g for 20 seconds. *Optional:* Resuspend the pellet by vortexing and pass the flow-through through the same filter one additional time to improve yield.
5. Discard the filter and resuspend the pellet by vigorous vortexing for 10 seconds. Centrifuge at 600 × g for 4 minutes to collect the nuclear pellet. Transfer the supernatant to a new microfuge tube on ice and proceed to **Step 9**.
6. Resuspend the nuclear pellet in 0.8–1.0 mL of cold Buffer B and centrifuge at 1,000 × g for 5–8 minutes to remove membrane debris. Carefully remove and discard the supernatant completely. The remaining pellet contains isolated nuclei. If contamination by large organelles (e.g., mitochondria) is a concern, a modified Buffer B may be used (see Technical Notes below).
7. Resuspend the pellet in 0.8 mL of Buffer C and centrifuge at 600 × g for 5 minutes. Remove and discard the supernatant. *Optional:* The supernatant may be retained for troubleshooting purposes.
8. Resuspend the nuclear pellet in 17 µl of buffer C. Take 6 µl of this to count nuclei on a cell counter (you can use AO/PI or trypan blue at a 1:1 ratio). The remaining 10 µl of resuspended nuclei will be used for LC/MS analysis (see Tech Notes below).
9. Centrifuge the supernatant from step 5 at 16,000 × g for 20 min. Transfer supernatant to a new tube (this is the cytosolic fraction for metabolite analysis).

Protocol for Cultured Cells

(Pre-chill buffers on ice)

1. Collect 10–40 × 10^6 cultured cells by low-speed centrifugation at 500 × g for 5 min. Wash the cell pellet once with 1 mL cold PBS and remove the supernatant completely.
2. Resuspend the cell pellet in 500 µl cold buffer A and incubate on ice for 8–10 min. After incubation, vortex the tube vigorously for 20–30 seconds. Transfer the cell suspension to a filter cartridge with a collection tube.
3. Centrifuge in a tabletop microcentrifuge at ~16,000 × g for 20 s. Resuspend the pellet by pipetting up and down several times, then pass the suspension through the filter cartridge one additional time.
4. Proceed with Steps 5–9 of the mammalian tissue protocol to isolate cytosolic and nuclear fractions.

Tech Notes

1. Methanol extraction for metabolites:
   - For isolated nuclei (10 µL in Buffer C), mix with 90 µL ice-cold methanol.
   - For the cytosolic fraction, mix 100 µL of the cytosolic fraction with 400 µL ice-cold methanol.

2. Nuclear samples undergo three freeze–thaw cycles using liquid nitrogen. Sonication in an ice-water bath is performed between freezing cycles to disrupt the nuclear membrane. Samples are then incubated at −20 °C for 3 h to precipitate proteins, followed by centrifugation at 16,000 × g for 15 min at 4 °C. Supernatants are transferred to new tubes, dried in a SpeedVac at −20 °C, and stored at −80 °C. Prior to analysis, samples are reconstituted in 100 µL acetonitrile/water (1:1, v/v) for LC-TQMS analysis.
3. It is recommended to use Buffer A as a cytosolic blank and Buffer C as a nuclear blank through the same methanol extraction workflow.
4. If mitochondrial contamination is a concern, Buffer B may be replaced with modified Buffer B in Step 6 (e.g., add 10 µL of 10% NP-40 in ddH_2O to 1 mL Buffer B and mix well) for resuspension of the nuclear pellet. When using modified Buffer B, perform two washes with 0.5 mL Buffer C in Step 7.
5. This kit has been validated using rat liver tissue and human cell lines. For hard tissues such as skeletal muscle, adding 50–60 mg of tissue dissociation beads to the tissue slurry may increase nuclear yield in Step 2.