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## Minute<sup>TM</sup> Detergent-Free Nuclei Isolation Kit (Non-Sterile)

Catalog number: NI-024

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### Description

The detergent-free nuclei isolation kit is designed to rapidly isolate intact nuclei from animal cultured cells or tissues (fresh or frozen). Intact nuclei can be isolated from the samples using proprietary spin-column-based technologies in less than 20 min without using tissue homogenizer and any detergents. The traditional method for nuclei isolation involves the use of non-ionic detergent, which has a tendency to cause unwanted nuclear aggregation. It is unclear why some nuclei are aggregated while others are not, and it is also unknown whether non-aggregated nuclei are an unbiased representation of the whole nuclei population. Detergents also have the potential to damage the nuclear envelope resulting in leakage of nuclear matrix materials. For some cell/tissue types, nuclear envelop proteins could also be stripped off by the detergents. Minute<sup>TM</sup> Detergent-Free Nuclei Isolation Kit provides a whole new way for nuclei isolation compared to traditional methods.

**How it works:** cells/tissues are first sensitized by buffer A before passing through the proprietary filter in a zigzag manner when high-speed centrifugal force is applied. The cells are ruptured when passing through the filter, leaving intact native nuclei in the flow-through. The nuclei are separated from other small cell debris by low-speed centrifugation using the proprietary buffer B. The native and intact nuclei isolated can be used for various downstream applications that include but are not limited to: FACS analysis, single nucleus analysis (such as RNA-seq and ATAC-seq), immunofluorescence staining, cell cycle analysis, and apoptosis research.

### Kit components

1. 15 ml buffer A
2. 30 ml buffer B
3. 20 protein extraction filter cartridges
4. 20 collection tubes
5. Plastic rod (2)

**Shipping:** This kit is shipped at ambient temperature.

**Storage:** Store Buffer B at -20°C and the rest of the kit at 4°C.

### Additional Materials Required

Tabletop Microcentrifuge with >14,000 rpm (16,000 X g)

### Important information

This kit, in general, can be used for the isolation of nuclei from most animal cultured cells and tissues (fresh or frozen). However, the purity and integrity of isolated nuclei are cell/tissue type-dependent. A



typical yield of intact nuclei from mouse liver or kidney tissue is 1-2 million /20mg. The output from other tissues or cells may vary depending on the percentage of ruptured cells after passing the spin column (typically 70-95%). Fresh tissues may be frozen at -20oC for at least 30 minutes (and thaw out on ice) if less than 70% of the cells are ruptured or lysed. RNase inhibitors should be added to an aliquot of buffer A and buffer B if isolated nuclei are intended for RNA-related applications such as single nucleus RNA sequencing.

### **Nuclei Isolation Protocol for Cultured Cells: (Pre-chill buffers on ice)**

1. Collect 10-50 million cultured cells by low-speed centrifugation (500 X g for 5 min). Wash the cell pellet once with 1 ml cold PBS. 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 microfuge at ~16,000 X g for 20 seconds, resuspend the pellet by pipetting up and down a few times, and re-pass the cells through the filter one more time.
4. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 500 X g for 2 to 4 min (smaller nuclei require longer centrifugation time). Remove and discard the supernatant.
5. Resuspend the nucleus pellet in 0.5-0.8 ml cold buffer B, then centrifuge at 600 X g for 8-10 min to remove membrane debris. The pellet contains isolated nuclei.

### **Nuclei Isolation Protocol for Mammalian Tissues: (Pre-chill buffers on ice)**

1. Add 20-30 mg fresh or frozen **soft tissue** to the filter cartridge. For frozen tissues, thaw them out completely on ice. **For muscle tissues**, place the tissue on the surface of a clean glass or plastic plate, and mince the tissue with a sharp blade into tissue slurry or past. Transfer the tissue to a filter cartridge.
2. Add 200 µl cold buffer A to the filter, grind the tissue for about 1-2 min using the plastic rod provided (the plastic rod is reusable, clean by water or 70% ethanol). Double the grinding time for muscle tissue.
3. Add 300 µl cold buffer A to the same filter, incubate on ice for 5-10 min with the cap open. Then cap the tube and resuspend the tissue homogenate by inverting the tube a few times.
4. Centrifuge in a tabletop microfuge at 16,000 X g for 20 seconds (optional: resuspend the pellet by vortexing and re-pass the pass-through through the same filter one more time).
5. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 500 X g for 2-3 min. Discard the supernatant.
6. Resuspend the pellet in 0.5-0.8 ml cold buffer B, centrifuge at 600 X g for 8-10 min (to remove membrane debris). The pellet contains isolated nuclei.

### **Further Cleanup of Isolated Nuclei:**



For most samples, the isolated nuclei are clean enough for downstream applications. Further cleanup may be considered if contamination of cellular debris is a concern. The following protocol can be used for cleanup:

1. Resuspend nuclei pellet from above procedures in 100-200  $\mu$ l buffer B.
2. Add 10-20  $\mu$ l 1% NP-40 to the nuclear suspension (final NP-40 concentration is 0.1%). Mix well and incubate on ice for 3-4 min.
3. Centrifuge at 800 X g for 5 min. The pellet contains cleaner nuclei.

***NOTE: This step is application dependent. It may be beneficial if the exposure of detergent to isolated nuclei is not a concern (such as ATAC-seq). It is not recommended for single nucleus RNA-seq for reasons mentioned in the description section above. Please note that due to variations in lipid composition of isolated nuclei from different tissues, cleanup by NP-40 may cause lysis of the nuclei.***

#### **Storage of Isolated Nuclei:**

Isolated nuclei can be resuspended in a tissue culture medium that contains 5-10% FBS or BSA and stored at 4°C for a few days without significant change in morphology. For long-term storage or transportation, **Minute™ Anti-Clumping Nuclei Storage Buffer (Cat# WA-014)** is recommended. Alternatively, resuspend the nuclei in 0.5 ml buffer B and store them at -70 - 80°C.