Minute™ Single Nucleus Isolation Kit for Tissues/Cells

Catalog number: SN-047

Description

Isolation and purification of nuclei from tissues and cultured cells are common lab practice. Isolated nuclei, especially single nucleus, are widely used for a variety of experiments such as FACS analysis, single nucleus analysis (such as RNA-seq and ATAC-seq), immunofluorescence staining, cell cycle analysis and apoptosis research. Traditional method for isolation of nuclei is relatively simple and straight forward. However, nucleus aggregation and formation of high percentage of doublets are frequently encountered problems. This kit is specially designed to address these issues. The protocol is simple, rapid and very effective. High percentage of well separated single nucleus can be obtained in about 30 min. In comparison to traditional method the kit requires less starting material and has a larger range of sample size (1-30 mg). The buffers contain proprietary mix of detergents for efficient cell lysis. If the presence of detergent is not desired, a detergent-free nuclei isolation kit is also available under cat# NI-024.

Kit components

1. Lysis buffer 15 ml
2. Washing buffer 20 ml
3. Filter cartridges /collection tubes 20
4. Pestle for 1.5 ml microfuge tube 2

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

Additional Materials Required

Table-Top Microcentrifuge, 1 X PBS and 1 X PBS with 5% BSA

Important information

This kit can be used for isolation of nuclei from fresh/frozen tissues and cultured cells. However, the purity and integrity of isolated nuclei are depending upon the tissue/cell types. Generally, soft tissue such as liver and kidney will show higher yield and purity than hard tissues such as skeletal muscle and cardiac muscles. All centrifugation step can be performed at room temperature. Please read the protocol carefully prior to experiment.

Protocol for Tissues:
(Pre-chill buffers on ice)

1. Add 1-25 mg fresh or frozen tissue to a 1.5 ml Eppendorf tube followed by addition of 200 µl cold lysis buffer. Homogenize the tissue using the pestle provided by grinding gently with twisting force for 2-3 min (the pestle is re-usable, clean it with alcohol and air dry).
2. Add 400 µl cold lysis buffer to the same tube and continue to grind for a few more times. Cap the tube and incubate on ice for 5-10 min. After incubation, pour all cell lysate into a filter in a collection tube.
3. Cap the filter and immediately centrifuge at 600 X g for 5 min. Discard the filter, carefully remove and discard the supernatant. Resuspend the pellet in 0.5-0.8 ml cold washing buffer by pipetting up and down for 20-30 times.
4. Centrifuge at 500 X g for 5 min. Remove and discard the supernatant. Resuspend the pellet (isolated nuclei) in 50-200 µl PBS containing 5% BSA or other buffer of your choice. Be sure to rinse the wall of the tube to get all nuclei.

Protocol for Cultured Cells:
(Pre-chill buffers on ice)

1. Collect 0.2-10 million cultured cells by low speed centrifugation (600 X g for 5 min). Wash the cell pellet once with 1 ml cold PBS. Remove the supernatant completely. Add 200 µl buffer A to the tube and grind with the pestle provided for 20-30 times. Add another 400 µl Buffer A to the tube and incubate at 4^{o}C for 5-10 min. After incubation, pour all cell lysate into a filter in a collection tube. Follow step 3 to step 5 above.

Tech note:

1. Though as small as 1 mg tissue and 200,000 cells can be used, we recommend to use 15-20 mg tissue and 4-5 million cultured cells/sample if starting material is not a limiting factor.
2. The yield and purity of nuclei are sample type-dependent. For some samples such as muscle tissues the yield is lower than soft tissues. If muscle tissue or tissue rich in connective tissue such as intestines are used, place the tissue on the surface of a clean glass or plastic plate, mince the tissue with a sharp blade into tissue slurry or past and place it in an Eppendorf tube.
3. A typical yield of intact nuclei from cortex is about 1 million/10 mg tissue. If very small amount of starting tissue or cells are used, the nuclei pellet may not be visible.
4. If there is liquid retention in step 3, the g force can be increased to 800 X g for 5 min. This indicates that excessive starting material has been used. It should be reduced by half in subsequent experiment. Don't use more than 30 mg tissue/sample.

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