Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells

Catalog Number: BN-020

Description

Isolated nuclei are widely used for various experiments such as FACS, single nucleus analysis (such as RNA-seq and ATAC-seq), immunofluorescence staining, cell cycle, and apoptosis research. Single-cell RNA-seq is a powerful technology for studying the complex cellular composition of tissues. However, Neurons are highly interconnected, and it is challenging to obtain single cells from neuronal tissues such as the brain and spinal cord. It is even more difficult to isolate intact cells from frozen neuronal tissues. Due to these limitations, single-cell RNA-seq is being substituted by single-nucleus-seq. The traditional method for single nucleus isolation from neuronal tissue is relatively tedious and time-consuming, and the yield is usually low because it is difficult to get rid of contaminating myelin and other cellular components. This kit is designed to address the issues with a simple, rapid, and effective protocol. The highly purified single nucleus can be obtained in about 30 min. Compared to the traditional method, the kit requires much less starting material and can handle an extensive range of sample sizes (1-25 mg). The buffers contain a proprietary mix of detergents for efficient cell lysis. If the presence of detergent is not desirable, a detergent-free nuclei isolation kit is also available under **Cat# NI-024**.

Kit components (20 Preps):

1.	Buffer A	15 ml
2.	Buffer B	25 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 4ºC**.**

Additional Materials Required: Table-Top Microcentrifuge and 1 X PBS with 5% BSA

Important information:

This kit can be used for isolation of nuclei from most neuronal tissues/cultured cells (fresh or frozen). However, the purity and integrity may vary. Generally grey matter of brain and cultured cells will give higher yield and purity than white matter and spinal cord. All centrifugation steps can be performed at room temperature. Please read tech note below before performing the experiment. If isolated nuclei are used for RNA related work, add RNase inhibitors (such as RNasin Plus) to aliquot of buffer A and B prior to use.

Protocol

Note: Sample size range: brain tissue 1-80 mg, spinal cord 1-50 mg; Pre-chill buffers on ice.

1. Add 20-30 mg fresh or frozen tissue to an Eppendorf tube followed by addition of 200 µl cold buffer A. homogenize the tissue using the pestle provided by grinding gently with twisting force for 50-60 times (the pestle is re-usable, clean it with alcohol and air dry).

2. Add 500 µl cold buffer A to the tube and continue to grind for 20-30 times. Incubate the tube on ice for 5 min and carefully transfer homogenate to a filter in collection tube (avoid larger debris that sink to the bottom of the tube). Incubate the tube with cap open at -20℃ for 5-10 min.
3. Cap the filter and immediately centrifuge at 13,000 X g for 30 seconds. Discard the filter and resuspend the pellet by pipetting up and down gently for 10-20-times (try to avoid lipids that attach to the wall of the tube). If there is a liquid retention in the filter reduce the amount of starting material by half.
4. Centrifuge at 600 X g for 5 min. Pour out the supernatant, resuspend the pellet (this is isolated nuclei, in many cases the pellet may not be obvious) in 200 µl PBS with 5% BSA that will be overlaid on top of buffer B in next step.
5. Add 1 ml cold buffer B to a 1.5 ml Eppendorf tube (remove bubbles if presence). Carefully overlay 200 µl nuclear suspension from step 4 on top of buffer B by slowly expel the nuclear suspension against wall of the tube. Centrifuge the tube at 1000 X g for 10 min. After centrifugation, cellular debris, oil and myelin will stay on the top (white-milky layer). The purified nuclei are found in pellet. Carefully remove the milky layer by withdrawing it into a 1 ml pipette tip and discard. Pour out the remining buffer B. Resuspend the pellet in 50-200 µl PBS containing 5% BSA or other buffer of your choice. Be sure to rinse the wall of the tube to collect all nuclei.

Protocol for Cultured Neuronal Cells:

1. Collect 0.1-10 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 pour all cell lysate into a filter in a collection tube. Incubate at -20ºC for 5-10 min. After incubation follow step 3 to step 5 above.

Tech note:

1. Though the kit can handle wide range of sample size, we recommend using 10-30 mg tissue and 2-5 million cultured cells/sample if starting material is not a limiting factor. Higher starting material yields more nuclei.
2. The purity of nuclei is sample type-dependent. For some samples such as grey matter of brain and some cultured cells, the nuclei isolated are relatively clean and cleanup by buffer B may not be necessary. An optional 0.5 ml PBS with 5% BSA wash of the pellet in step 4 may be sufficient. If spinal cord or white matter is used, cleanup by buffer B is recommended.
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. Cleanup by buffer B could cause further loss of nuclei.
4. If significant loss of nuclei is observed after buffer B clean up, the centrifugal force can be increased to 1,000-1,500 X g for 10 min in step 5. The bottom line is to use a centrifugal force that gives the maximum recovery of nuclei without significant contamination by myelin.
5. Due to the presence of non-ionic detergents in the buffers, some nuclear clumping is expected. If significant clumping is observed, the following steps may help improve the results:

   A. Resuspend the final nuclei pellet in 0.2-0.5 mL of PBS-BSA (1× PBS with 5% BSA), and pass the suspension through a 40 µm cell strainer using gravity flow to remove nuclear aggregates.
   B. Dilute Buffer A 1:1 with PBS-BSA and omit the -20 °C incubation step in Step 2.
   C. In some cases, reducing the starting material by half may also help reduce clumping.