Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells

Catalog number: BN-020

Description

Isolated nuclei 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. Single cell RNA-seq is a powerful technology for studying complex cellular composition of tissues. However, Neurons are highly interconnected and it is very difficult to obtain single cells from neuronal tissues such as 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. 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 specially designed to address the issues with a protocol that is simple, rapid and effective. Highly purified single nucleus can be obtained in about 30 min. In comparison to traditional method, the kit requires much less starting material and can handle a large range of sample size (1-25 mg). The buffers contain 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

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 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 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.

Protocol for Brain, Spinal Cord and other Neuronal 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 buffer A. 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 buffer A to the tube and continue to grind for a few more times. Incubate the tube with cap open at -20°C for 15-20 min. After incubation, pour all cell lysate into a filter in a collection tube.
3. Cap the filter and immediately centrifuge at 800 X g for 5 min. Carefully remove and discard the supernatant. Resuspend the pellet in 0.5 ml cold PBS by pipetting up and down for 20-30 times.
4. Centrifuge at 600 X g for 5 min. Remove and discard the supernatant, resuspend the pellet in 200 ul PBS 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. Carefully overlay 200 µl nuclear suspension from step 4 on top of buffer B by slowly expel the liquid against wall of the tube. Centrifuge the tube at 800 X g for 10 min. After centrifugation, cellular debris 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 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 get all nuclei.

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

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 incubate at -20°C for 15-20 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 100,000 cells can be used, we recommend using 10-20 mg tissue and 2-5 million cultured cells/sample if starting material is not a limiting factor.
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. If spinal cord or white matter is used, cleanup by buffer B is required.
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 there is liquid retention in step 3, the g force can be increased to 1000 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 25 mg tissue/sample.