Minute™ Single Nuclei Isolation Kit for Plant Tissues

Catalog number: PS-054

Description

Single nuclei have become the ideal materials for next-generation genomics and proteomics. However, isolating high-quality single nuclei from plant tissues remains challenging. Most lab-developed methods are limited to specific tissue or species, and no standard protocol and buffer system exist to handle all tissue types from different species. Significant optimization may be needed for different samples. One major drawback of traditional methods is the clumping or aggregation of isolated nuclei, making them difficult for applications such as single nucleus RNA-seq and ATAC analysis. This kit is designed to rapidly isolate well-separated single nuclei from tissues of model and widely-studied plants with minimal clumping using a specially designed spin column and proprietary buffers. The protocol takes about 30 minutes and requires only 150-200mg of the starting material.

Kit components(20 preps):

1.	Buffer A	25 ml
2.	Buffer B	20 ml
3.	Buffer C	10 ml
4.	Filter cartridges	40
5.	2.0 ml Collection tube	20
6.	Pestle	2

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

Additional Materials Required

Table-Top Microcentrifuge
1.5 ml microfuge tube

Important information

Isolated nuclei may be used directly for downstream applications such as nucleic acid extraction and single nucleus analysis (snRNA-seq and ATAC-seq). Please read the protocol carefully before the experiment. If isolated nuclei are used for RNA-related applications, add RNase inhibitors to aliquots of buffers before use; for example, add RNAsin plus to the buffers to a final concentration of 200 U/ml.

Protocol

(Pre-chill buffers on ice and perform centrifugation at 4-8ºC.)

1. Weight 150-200 mg of fresh young plant tissues (leaf and seedlings are the most common tissue types). Fold and roll the tissue and insert it into a filter cartridge in a 2.0 ml collection tube provided. Add 200

µl buffer A to the filter and punch the sample in the filter repeatedly with a 1 ml pipette tip about 100-200 times to reduce the volume (this step takes about 1-2 min).

2. Grind the tissue with the flat end of the pestle using twisting force against the surface of the filter about 100-200 times (2-3 min). Pestle is reusable; for cleaning, rinse with water and dry with a paper towel.

3. Add an additional 400 µl buffer A to the filter and stir with a 200 µl pipette tip a few times. Cap the filter and centrifuge at 2,000 X g for 5 min.

4. Discard the filter and decant the supernatant. Resuspend the pellet in 0.5 ml buffer A by pipetting up and down 15-20 times. Centrifuge at 1,500 X g for 5 min, and remove the supernatant. Resuspend the pellet in 100 µl buffer A by pipetting, followed by adding 1 ml buffer B, and mix by gently pipetting a few times. Incubate on ice for 5-10 min.

5. Centrifuge at 200 X g for 2 min. Pour supernatant into a fresh 1.5 ml tube and centrifuge at 1,000 X g for 5 min. Resuspend the pellet in 400 µl buffer C and transfer to a filter cartridge in a 1.5 ml microfuge tube (not provided). Centrifuge at 200 X g for 2 min, then at 800 X g for 4 min with the cap open. Discard the filter, remove 200-250 µl supernatant and resuspend the pellet in the remaining supernatant by gentle pipetting. Alternatively, the supernatant can be removed entirely and resuspend the pellet in 100-200 µl of a buffer that is compatible with downstream experiments. In some cases, the nuclear pellet is semi-transparent and may not be seen easily.

Tech notes:

1. This kit is designed to isolate single nuclei from fresh plant leaves and soft tissues. The purity and integrity of isolated nuclei depend upon the tissue types.

2. We have tested this kit on leaves of *A. thaliana*, *S. viridis*, spinach, maize, soybean, apple tree, and rape with similar results. However, the yield and purity of isolated nuclei could vary significantly due to vastly diversified structural variations of different plant species.

3. Young and fresh plant leaves or soft roots are recommended as starting materials. Isolated nuclei can be visualized by trypan blue or DAPI staining using a light microscope and fluorescence microscope, respectively.

4. This kit is not suitable for mucilaginous plants.