

Hyperactive Plant Nuclear Extraction Kit

NH101



Instruction for Use
Version 25.1

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For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Hyperactive Plant Nuclear Extraction Kit enables rapid and efficient isolation and purification of nuclei from a wide range of plant tissues using density gradient centrifugation. This kit preserves nuclear membrane integrity while reducing cellular debris and secondary metabolites, and can be completed in approximately 50 min. The isolated nuclei is suitable for downstream epigenetic applications, including ATAC-Seq, CUT&Tag, and CUT&RUN. All reagents provided in the kit have undergone rigorous quality control and functional testing to ensure optimal stability and reproducibility of library preparation.

02/Components

Components	NH101-01 (12 rxns)
Lysis Buffer	24 ml
NPB-A	6 ml
NPB-B	6 ml
NPB-C	6 ml
Wash Buffer	12 ml

▲ NPB-A = Nuclei Purification Buffer A; NPB-B = Nuclei Purification Buffer B; NPB-C = Nuclei Purification Buffer C.

03/Storage

Store at 2 ~ 8°C and ship on ice pack.

04/Applications

It is compatible with a wide range of fresh or frozen plant samples. It has been validated using fresh/frozen *Triticum aestivum*, fresh *Arabidopsis thaliana*, fresh/frozen *Oryza sativa*, fresh *Glycine max* leaves, stems and roots, and frozen *Brassica napus* leaves. Young tissues such as juvenile leaves, root tips, and tender stems are recommended as the preferred starting material. Highly viscous tissues (e.g., fruits) may require pretreatment.

05/Self-prepared Materials

Mortar and pestle, spatula, liquid nitrogen, RNase-free tubes (1.5 ml and 2.0 ml), 70 µm cell strainer, 40 µm cell strainer, and protease inhibitor.

It is recommended to use Roche Complete Protease Inhibitor EDTA-Free Tablets (Sigma-Aldrich #5056489001) or a general-purpose protease inhibitor cocktail (Beyotime #P1005; 100 ×).

06/Notes

1. For nucleus-derived RNA applications, add an RNase inhibitor (final concentration $\geq 1,000$ U/ml) and DTT (final concentration 2 mM) to all reagents.
2. For muclage-rich plant samples, it is recommended to reduce the input amount or increase the volume of Lysis Buffer.
3. Steps of nuclei isolation following liquid nitrogen grinding should be performed on ice.

07/Experiment Process

Step 1: Sample Pretreatment

1. Mortar and pestle precooling: Place the pestle and spatula into the mortar, then pour liquid nitrogen into the mortar until the nitrogen can be added without splashing.
2. Tissue grinding: Place approximately 200 mg of plant tissue into the pre-chilled mortar containing liquid nitrogen. Gently break the tissue into small pieces using the pestle. Before the liquid nitrogen fully evaporates, grind the tissue into powder. Transfer the powder using the pre-chilled spatula into a pre-chilled 2 ml RNase-free tube. If liquid nitrogen remains in the tube, leave the tube open until it fully evaporates.
 - ▲ When grinding multiple samples, keep the tubes containing the powder on dry ice or in liquid nitrogen until use.
 - ▲ The tissue input amount can be adjusted as needed. Grind quickly to prevent complete evaporation of liquid nitrogen, which may lead to sample freeze-thaw or degradation.
 - ▲ Over-grinding may increase background debris and compromise nuclear integrity. Stop grinding once the tissue becomes powder.

Step 2: Nuclei Extraction

Two options for nuclei isolation are provided:

Option One: Crude Extraction

- ▲ Crude extraction yields abundant nuclei but with reduced purity and increased impurities. It is suitable for applications that prioritize yield over purity, such as CUT&Tag and CUT&RUN.
1. Add 1 ml of Lysis Buffer to the ground tissue. Mix well by inverting and incubate on ice for 5 min.
 - ▲ (Optional) Add 50 $\times$ protease inhibitors to the Lysis Buffer to a final concentration of 1 $\times$.
 - ▲ 50 $\times$ Protease Inhibitor Preparation: Dissolve one protease inhibitor tablet (Sigma-Aldrich #5056489001) in 1 ml of ddH₂O and store at -30 $\sim$ -15°C.
 2. Pass the solution from last step sequentially through 70 μ m and 40 μ m cell strainers to remove large debris, then transfer the supernatant to a new 2.0 ml tube.
 3. Centrifuge at 900 rpm (80 $\times$ g) for 5 min at 4°C, then transfer the supernatant to a new 1.5 ml tube.
 - ▲ Avoid disturbing the pellet, which contains cell debris.

4. Centrifuge at 2,300 rpm ($500 \times g$) for 5 min at 4°C, then discard the supernatant. Keep a small amount of supernatant to avoid aspirating the pellet.
5. Add 500 µl of Lysis Buffer and resuspend the nuclei by pipetting. Centrifuge at 2,300 rpm ($500 \times g$) for 5 min at 4°C, then discard the supernatant.
6. Add 500 µl of Lysis Buffer and resuspend the nuclei by pipetting. Centrifuge at 2,300 rpm ($500 \times g$) for 5 min at 4°C, then discard the supernatant.
▲ This step is necessary for mature plant tissues but can be skipped when processing young tissues.
7. Add 500 µl of Wash Buffer and resuspend the nuclei by pipetting. Centrifuge at 2,300 rpm ($500 \times g$) for 5 min at 4°C, then discard the supernatant.
8. Add 200 - 400 µl of Wash Buffer and resuspend the nuclei by pipetting. Take 1 - 5 µl of the nuclei suspension and assess nuclear membrane integrity and count nuclei by DAPI staining under a fluorescence microscope or by trypan blue staining under a standard light microscope.
▲ Dilute 1 - 5 µl of the nuclear suspension 2 - 5-fold before DAPI staining to ensure accurate counting.
9. Centrifuge the remaining nuclear suspension from step 8 at 2,300 rpm ($500 \times g$) for 5 min at 4°C. Discard the supernatant to obtain the nuclear pellet.
▲ It is recommended to proceed with downstream experiments as soon as possible to maintain data quality.

Option Two: Refined Extraction

▲ Refined extraction yields fewer nuclei but with higher purity and reduced contamination. It is suitable for assays that prioritize yield over quantity, such as ATAC-Seq.

1. Add 1 ml of Lysis Buffer and mix well by inversion. Incubate on ice for 5 min.
▲ (Optional) Add $50 \times$ protease inhibitors to the Lysis Buffer to a final concentration of $1 \times$.
2. Pass the solution from last step through 70 µm cell strainer to remove large debris, then transfer the supernatant to a new 2.0 ml RNase-free tube.
3. Centrifuge at 2,300 rpm ($500 \times g$) for 5 min at 4°C, then discard the supernatant. Keep a small amount of supernatant to avoid aspirating the pellet.
4. Add 500 µl of NPB-A and resuspend the nuclei by pipetting. Pass the solution through 40 µm cell strainer, transfer the supernatant to a new 2 ml RNase-free tube.
5. Slowly add 500 µl of NPB-B to the bottom of the tube from last step. The solution will separate into two layers. Then slowly add 500 µl of NPB-C to the bottom of the tube, resulting in three layers. Centrifuge at 4,600 rpm ($2,000 \times g$) for 20 min at 4°C.
▲ Add the reagents slowly and gently. Do not force any residual liquid remaining in the pipette tip into the tube, as the disturbance may disrupt layer formation.
▲ (Optional) Alternatively, 500 µl of NPB-C can first be transferred into a new 2.0 ml RNase-free tube. Then slowly add 500 µl of NPB-B along the tube wall so that it overlays the NPB-C layer. Slowly layer the suspension from step 4 (NPB-A) along the NPB-B layer, resulting in three layers. Centrifuge at 4,600 rpm ($2,000 \times g$) for 20 min at 4°C.

6. The intermediate layer (NPB-B) contains the nuclei (see Fig 1). Carefully discard the top layer (NPB-A), and transfer the middle layer to a 1.5 ml RNase-free tube, while leaving the bottom layer (NPB-C) undisturbed.

▲ If white debris is observed in the intermediate layer (NPB-B), carefully aspirate and discard it.

7. Add 500 μ l of Wash Buffer to the intermediate layer from step 6 and mix well by pipetting. Centrifuge at 2,300 rpm ($500 \times g$) for 5 min at 4°C, then discard the supernatant.

8. Add an appropriate amount of Wash Buffer, and resuspend the nuclei by pipetting. Take 1 - 5 μ l of the nuclei suspension and assess nuclear membrane integrity and count nuclei by DAPI staining under a fluorescence microscope or by trypan blue staining under a standard light microscope (see Fig 2).

▲ If no pellet is visible, resuspend in 50 μ l of Wash Buffer. If a visible pellet (approximately 1 - 2 mm) is present, resuspend in 400 μ l of Wash Buffer.

9. Centrifuge the remaining nuclear suspension from step 8 at 2,300 rpm ($500 \times g$) for 5 min at 4°C. Discard the supernatant to obtain the nuclear pellet.

▲ It is recommended to proceed with downstream experiments as soon as possible to maintain data quality (see Fig 3).

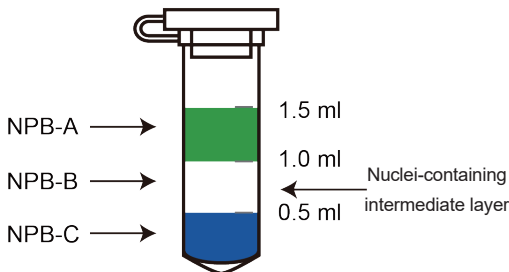


Fig 1. Schematic of Three-Layer Density Gradient Centrifugation

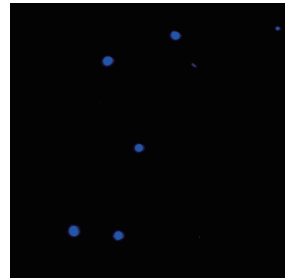


Fig 2. DAPI Staining of *Triticum aestivum* Leaf Nuclei

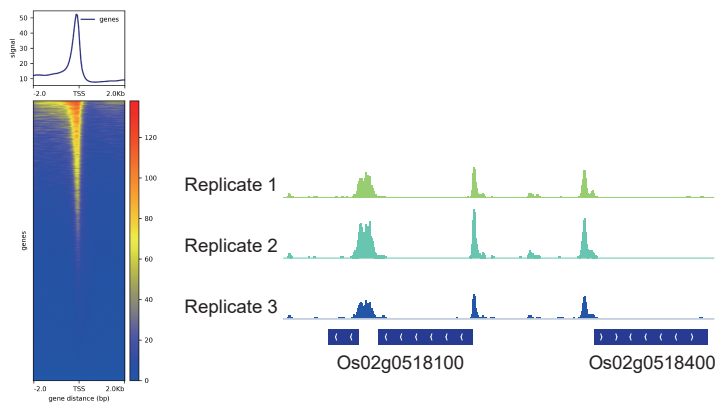


Fig 3. ATAC-Seq Signal Profiles of *Oryza sativa* Leaf Nuclei



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