Hyperactive Plant Nuclear Extraction Kit

NH101

Version 24.1

Product Description

Hyperactive Plant Nuclear Extraction Kit is specifically designed for nuclei extraction from various plant samples using density gradient centrifugation. The kit preserves nuclear membrane integrity while minimizing contamination from cellular debris and secondary metabolites. The entire procedure can be completed within approximately 50 minutes, and the purified nuclei are suitable for ATAC-Seq. All components in the kit have undergone rigorous quality control and functional testing to ensure optimal stability and repeatability.

Components

Components	NH101-01 (12 rxns)
Lysis Buffer	12 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.

Storage

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

Applications

The kit is designed for nuclei extraction from various fresh or frozen plant samples (e.g., wheat, *Arabidopsis thaliana, Oryza sativa,* soybean, *Brassica napus*) and has been optimized for young plant tissues such as young leaves, root tips, and tender stems. Mucilaginous tissues (e.g., fruits) may require additional pre-processing steps.

Self-prepared Materials

Pestle, mortar, spatula, liquid nitrogen, RNase-free tubes (1.5 ml and 2.0 ml), cell strainer (70 μm and 40 μm), Roche Complete Protease Inhibitor EDTA-Free Tablets (Sigma-Aldrich #5056489001).

Notes

1. If downstream applications are RNA-related experiments, all reagents must contain RNase inhibitors (final concentration >1,000 U/ml) and DTT (final concentration of 2 mM).
2. It is recommended to reduce the initial sample amount or increase the usage amount of the Lysis Buffer for mucilaginous plant samples.
3. After liquid nitrogen grinding, all steps for nuclei extraction must be performed on ice to maintain nuclear integrity.

Experiment Process

1. Pre-cool mortar and pestle: Pour liquid nitrogen into the mortar to pre-cool the mortar, pestle, and spatula. Repeat until no splashing occurs when adding fresh liquid nitrogen.
2. Tissue grinding
   a. Place approximately 200 mg of plant tissue into the pre-cooled mortar containing liquid nitrogen.
   b. Crush the tissues gently into small pieces using a pestle.
   c. As the liquid nitrogen is nearly evaporated, quickly grind the tissue into fine powder.
   d. Transfer the fine powder to a pre-cooled 2 ml RNase-free tube using a pre-cooled spatula. (If residual liquid nitrogen remains in the tube, leave the tube uncapped until it fully evaporates before sealing.)
   ▲ For multiple samples: Place the tubes containing the ground sample on dry ice or in liquid nitrogen temporarily while processing subsequent samples.
   ▲ Optimization: Adjust the input of tissue for different samples (e.g., reducing it to 100 mg for mucilage-rich tissues). Grind rapidly to avoid thawing or degradation.

3. Add 50 × Proteinase Inhibitor to Lysis Buffer to a final concentration of 1 ×. Then add 1 ml of prepared Lysis Buffer to the tube and mix well by inversion. Incubate on ice for 5 - 10 min.

   ▲ Prepare immediately before use.

   ▲ Preparation of 50 × Protease Inhibitor: Dissolve one protease inhibitor tablet (Sigma-Aldrich #5056489001) in 1 ml of ddH_2O and store at -30 ~ -15°C.

4. Filtration: Pass the tissue suspension from step 3 through a 70 μm cell strainer to remove large debris and transfer the suspension to a new 2.0 ml RNase-free tube.

5. Centrifuge: Centrifuge at 2,300 rpm (500 × g) and 4°C for 5 min and carefully discard the supernatant, while leaving a small amount of supernatant to avoid disturbing the pellet.

6. Nuclear resuspension: Resuspend the nuclei with 500 μl of NPB-A, pass through a 40 μm cell strainer, and transfer the suspension to a new 2.0 ml RNase-free tube.

7. Density gradient purification:

   a. Slowly add 500 μl of NPB-B to the bottom of the tube from Step 6, at which point the solution separates into two layers.

   b. Slowly add 500 μl of NPB-C to the bottom, at which point the solution separates into three layers.

   c. Centrifuge at 4,600 rpm (2,000 × g) and 4°C for 20 min.

   Optional procedure:

   a. Add 500 μl of NPB-C to a new 2.0 ml RNase-free tube.

   b. Slowly add 500 μl of NPB-B along the tube wall onto the NPB-C layer.

   c. Add the suspension from Step 6 onto the NPB-B layer.

   d. Centrifuge at 4,600 rpm (2,000 × g) and 4°C for 20 min.

   ▲ Critical notes: Pipette slowly to avoid disrupting the layers and do not forcefully expel any residual liquid from the pipette tip.

8. Aspirate the nuclear layer: The interface between NPB-B and NPB-C is the nuclear layer (Fig 1. Schematic diagram of three-layer density gradient centrifugation). Aspirate 200 μl of nuclei suspension from this layer and transfer it to a new 1.5 ml RNase-free tube.

   ▲ If a white clump (debris aggregate) is observed at the interface, carefully aspirate and discard it before collecting the nuclei suspension.

9. Add 500 μl of Wash Buffer and mix by pipetting. Centrifuge at 2,300 rpm (500 × g) and 4°C for 5 min, and discard the supernatant.

10. Resuspend the nuclei in 50 - 100 μl of Wash Buffer. Nuclei counting and morphological observation can be performed using DAPI staining under a fluorescence microscope or trypan blue staining under an optical microscope, using 1 - 5 μl of the nuclei suspension (Fig 2. DAPI staining of wheat leaf nuclei).

11. Centrifuge the remaining nuclei suspension from step 10 at 2,300 rpm (500 × g) and 4°C for 5 min, and discard the supernatant to obtain the nuclei precipitate.

    ▲ It is recommended to proceed to the downstream experiments as soon as possible to ensure optimal experimental data collection (Fig 3. TSS enrichment and IGV view of ATAC-Seq from O. sativa leaf nuclei).

12. The procedure for the downstream experiment ATAC-Seq (Vazyme #TD711):

    a. Resuspend the nuclei in 50 μl of pre-cooled TW Buffer. Centrifuge at 2,300 rpm (500 × g) and 4°C for 10 min and discard the supernatant.

    ▲ If the nuclei exhibit minimal contamination or are present in small amounts, please skip this step and proceed to 08-3/Fragmentation and Termination in the Vazyme #TD711 manual.

    b. Proceed to 08-3/Fragmentation and Termination in the Vazyme #TD711 manual.

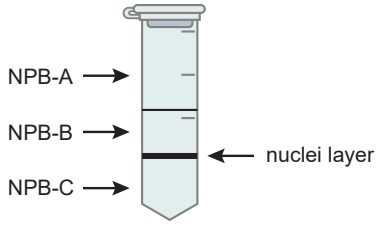

Fig 1. Schematic diagram of three-layer density gradient centrifugation

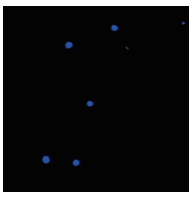

Fig 2. DAPI staining of wheat leaf nuclei

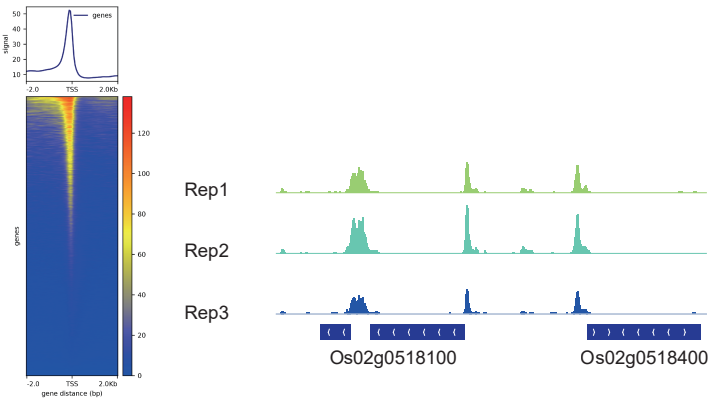

Fig 3. TSS enrichment and IGV view of ATAC-Seq from O. sativa leaf nuclei