Plant Nuclear Protein Extraction Kit

Cat. No. PNE-318K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit is for the rapid isolation of nuclei and extraction of functional nuclear proteins from plant leaves
Applications	The nuclear protein extract is suitable for the detection of DNA-protein interactions using gel-shift assay, DNase-I footprinting analysis as well as Western blot assay and similar techniques. The isolated nuclei can also be used as a source for chromatin, genomic DNA, RNA, etc.
Usage	1 Kit sufficient for 30 extractions (20 g of fresh or frozen leaves)
Storage	The kit is shipped on wet ice and is stored at 2-8 centigrade. The Filter Mesh 100 can be stored at room temperature. It can be reused and can be autoclaved.
Kit Components	Nuclei Isolation Buffer 4X (NIB) 500 ml Percoll 100 ml Sucrose 2.3 M 100 ml TRITONä X-100 10% solution 10 ml Extraction Buffer 10 ml Nuclei PURE Storage Buffer 10 ml Filter Mesh 100 4 each
Materials Required but Not Supplied	· Dithiothreitol (DTT) · Protease Inhibitor Cocktail for Plants · Test tubes (50 ml, 14 ml, 1.5 ml)

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	· Centrifuge (Sorvall with SS-34 rotor or equivalent) · Microcentrifuge (Eppendorf 5417R or equivalent) · Liquid nitrogen · Mortar and pestle · Funnel · Microscope (optional)
Separation Protocol	Perform all steps at 2-8 centigrade. Use pre-cooled buffers and equipment. Be sure that all the solutions are mixed thoroughly. All centrifugations should be performed at 2-8 centigrade with pre-cooled rotors. For better yield of nuclei and nuclear proteins it is recommended to use relatively young leaves. The yield of protein extract (per gram leaves) increases with the increase in initial leaf weight. Different species of the same plant can give different yields of nuclei and nuclear proteins. A. Cell Lysis The quantities given in the procedure are based on nuclei isolation/extraction from 20 grams of leaves. For different amounts, calculate accordingly. 1. Prepare 1X NIB by diluting the Nuclei Isolation Buffer 4X (NIB) 4-fold with deionized water. Mix well. Then add DTT to a final concentration of 1 mM. The DTT should be added fresh before every extraction. 2. Form the filter mesh into a conical shape and place it inside a suitable funnel. 3. Grind fresh or frozen leaves with liquid nitrogen to a fine powder using a mortar and pestle. Let the liquid nitrogen evaporate completely. The color changes from gray-white to dark green. It is recommended to transfer the powder from the cold mortar to a new container before addition of the buffer to prevent the buffer from freezing. Add 60 ml of 1X NIB buffer (3 ml/g of tissue) and mix well. Alternatively, grind the leaves in a blender or homogenizer together with the 1X NIB buffer. 4. Pass the suspension, gradually, through the filter mesh into 50 ml tube(s) and squeeze the filter to collect all the liquid. 5. Spin the 50 ml tube(s) for 10 minutes at 1,260 x g. 6. Decant the supernatant and resuspend the pellet completely in 2 ml of 1X NIB

buffer containing 1:100 (v/v) Protease Inhibitor Cocktail. This buffer is designated as NIBA. The pellet may be difficult to resuspend. If required, pool the pellets from all the tubes. Notes: · At this step, use of NIBA [1:100 (v/v) Protease Inhibitor Cocktail in 1X NIB buffer] is highly recommended. · When using smaller amounts of plant leaves initially, consider that a minimal volume of 0.5 ml of NIBA should be used to resuspend the pellet.

7. For cell membrane lysis, add the 10% TRITON X-100 solution to a final concentration of 0.3 % (according to the volume used to resuspend the pellet) and mix. Note: Each type of plant requires a different percentage of TRITON X-100 for proper lysis. Take several microliters of the cells in buffer and view them under the microscope. If a large proportion of cells remain intact, add more TRITON X-100, until most of the cells have lysed. If lysis of nuclei or a gelatinous mass is observed, lyse the cells with a lower final concentration of TRITON X-100. Suggested final percentages of TRITON X-100: Tobacco, Spinach, Arabidopsis: 0.3% , Tomato: 1%

B. Isolation of Nuclei Nuclei isolation can be performed to several degrees of purity (Crude, Semi-pure, and Highly-pure). There is an indirect correlation between the degree of nuclei purification and the resulting yield of nuclei and nuclear proteins. The Highly-pure Preparation of Nuclei procedure may often result in significant loss of nuclei for Arabidopsis and other plants with relatively small nuclei. To obtain optimal yield of nuclei and nuclear proteins, it is recommended to use the Crude Preparation of Nuclei and Semi-pure Preparation of Nuclei Procedures.

B1. Crude Preparation of Nuclei

a. Centrifuge lysate (section A, step 7) at 12,000 x g for 10 minutes in 1.5 ml tube(s).

b. Remove supernatant and wash the pellet(s) by resuspending in 1 ml of NIBA and centrifuge for 5 minutes at 12,000 x g. If required, pool the contents of all tubes to collect the pellet. At this stage the nuclei can either be stored without further manipulations or extracted for nuclear proteins. For storage, remove the supernatant and resuspend the nuclei pellet in 100 ml of Nuclei PURE Storage Buffer and store at -70 centigrade for up to three months. For nuclear protein extraction, proceed to section C.

B2. Semi-pure Preparation of Nuclei

a. Mix the 2.3 M sucrose solution well before use.

b. Apply the lysate (section A, step 7) on top of a 0.8 ml cushion of 2.3 M sucrose in 1.5 ml tubes (~0.6 ml of lysate in each tube). Note: For Arabidopsis, the 2.3 M Sucrose should be diluted to 1.5 M with 1X NIB buffer and used as a cushion instead of the 2.3 M Sucrose.

c. Centrifuge at 12,000 x g for 10 minutes.

d. Aspirate the upper green phase and the sucrose cushion layer without disturbing the pellet of nuclei.

e. Wash the pellet(s) twice, by resuspending in 1 ml of NIBA and centrifuging for 5 minutes at 12,000 x g. If required, pool the pellets from all tubes. At this stage the nuclei can either be stored without further manipulations or extracted for nuclear proteins. For storage, remove the supernatant and resuspend the nuclei pellet in 100 ml of Nuclei PURE Storage Buffer and store at -70 centigrade for up to three months. For nuclear protein extraction, proceed to section C.

B3. Highly-pure Preparation of Nuclei

a. Prepare “gradient isolation tube”-Dilute the Percoll to 60% with 1X NIB (1.8 ml of Percoll and 1.2 ml of 1X NIB). Put 3 ml of 2.3 M Sucrose in 14 ml tube. Carefully add 3 ml of the 60% Percoll solution. Two separate phases should be formed. Notes: For Arabidopsis, the Percoll layer should be diluted to 40 % with 1X NIB (1.2 ml of Percoll and 1.8 ml of 1X NIB). Scaling of this highly-pure nuclei isolation procedure can be performed using 50 ml “gradient isolation tubes” with 10 ml of each layer, sucrose and Percoll (for up-scaling), or 2 ml “gradient isolation tubes” with 0.7 ml of each layer, sucrose and Percoll, (for downscaling).

b. Carefully apply the lysate (section A, step 7) on top of the Percoll layer.

c. Centrifuge 30 minutes at 3,200 x g. Most of the nuclei are banded at the interface between the sucrose and the Percoll layers.

d. Gently collect the nuclei band with a Pasteur pipette and transfer into a new 14 ml tube. e. Wash the nuclei to remove Percoll and sucrose, by adding NIBA (2X the nuclei band volume) to the collected nuclei band and centrifuge for 5 minutes at 3,200

x g.
f. Resuspend the washed pellet in 1 ml of NIBA and transfer to 1.5 ml tube.
g. Centrifuge 12,000 x g for 5 minutes. At this stage the nuclei can either be stored without further manipulations or extracted for nuclear proteins. For storage, remove the supernatant and resuspend the nuclei pellet in 100 ml of Nuclei PURE Storage Buffer and store at -70 centigrade for up to three months. For nuclear protein extraction, proceed to section C.
C. Nuclear Protein Extraction
a. Prepare a Working Extraction Buffer by adding DTT to the Extraction Buffer to a final concentration of 5 mM and adding 1:100 (v/v) Protease Inhibitor Cocktail. The DTT and the Protease Inhibitor Cocktail should be added freshly before every extraction.
b. Thoroughly resuspend the nuclei pellet with the Working Extraction Buffer (2/3 of the pellet volume). If a higher protein concentration is required, reduce the volume of Working Extraction Buffer to the minimal volume needed for suspension.
c. Vortex at medium-high speed for 15-30 minutes at 4 centigrade.
d. Centrifuge for 10 minutes at 12,000 x g.
e. Transfer the supernatant to a clean chilled test tube. For storage, snap-freeze aliquots of the supernatant in liquid nitrogen and store at -70 centigrade.
D. Salt Removal The nuclear proteins extracted according to the procedure are suspended in a high salt buffer. Many downstream applications [EMSA (electrophoretic mobility shift assay), footprinting, and similar assays] require only small volumes of the nuclear protein extract and the high salt concentration is diluted by addition of the other components of the analysis. If, however, salts interfere with the downstream application, salt removal may be performed rapidly using Sephadex PD-10 columns. The recommended buffer for this procedure is 20 mM Hepes, pH 7.9, 1.5 mM MgCl2, 0.2 mM EDTA, 10 mM KCl, 25% (v/v) glycerol, and 1 mM DTT. The salts may also be removed by dialysis of the nuclear extracts against the preferred buffer.

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