Product Information

CelLytic™ PN Isolation/Extraction Kit

For plant leaves

CELLYTPN1

Product Description

The CelLytic™ PN kit is designed for the isolation of nuclei and extraction of functional nuclear proteins from plant leaves. Nuclei or nuclear proteins can be extracted from a few grams up to hundreds of grams of fresh or frozen leaves. The nuclear protein extract is suitable for the detection of DNA-protein interactions using gel-shift assays, DNase I footprinting analyses, as well as Western blot assays and similar techniques.[1-4] The isolated nuclei can also be a source for such material as chromatin, genomic DNA, histones, and nuclear RNA.

The nuclear protein extraction method includes cell wall breakage, followed by disruption of the cell membrane using a detergent.[5] The nuclei can be isolated and separated in different degrees of purity:[6]

- Crude
- Semi-pure on a sucrose cushion
- Highly-pure on a Percoll®/sucrose layer

The nuclear proteins are subsequently extracted from the nuclei with a high salt buffer.[1-3]

The CelLytic™ PN extraction kit has been tested for use with tobacco, tomato, spinach, and *Arabidopsis*. Several theses[7] and dissertations[8-10] have cited use of product CELLYTPN1 in their protocols.

Components

- Nuclei Isolation Buffer 4× (NIB): 500 mL
- Percoll®: 100 mL
- Sucrose, 2.3 M: 100 mL
- TRITON™ X-100 10% solution: 10 mL
- Extraction Buffer: 10 mL
- Nuclei PURE Storage Buffer: 10 mL
- Filter Mesh 100: 4 each

Reagents and Equipment Required

(Not provided)

Example Catalogue Numbers are given where appropriate.

- Dithiothreitol (DTT, such as Catalogue Numbers D9779 or 43816)
- Protease Inhibitor Cocktail for Plants (such as Catalogue Numbers P9599 or PIC0005)
- Test tubes (50 mL, 14 mL, 1.5 mL)
- Centrifuge
- Microcentrifuge
- Liquid nitrogen
- Mortar and pestle
- Funnel
- Microscope (optional)

Storage/Stability

The CELLYTPN1 kit is shipped on cooler packs ('wet ice'). Long-term storage at 2-8 °C is recommended.

The Filter Mesh 100 can be stored at room temperature. It can be reused and autoclaved.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

- Perform all steps at 2-8 °C.
- Use pre-cooled buffers and equipment.
- Be sure that all the solutions are mixed thoroughly.
- All centrifugations should be performed at 2-8 °C 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.

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 1× NIB by diluting the Nuclei Isolation Buffer 4× (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. Place the filter mesh 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 1× NIB buffer (3 mL per 1 g of tissue). Mix well.
   - Alternatively, grind the leaves in a blender or homogenizer together with the 1× NIB buffer.
4. Pass the suspension, gradually, through the filter mesh into 50 mL tube(s). Squeeze the filter to collect all the liquid.
5. Spin the 50 mL tube(s) for 10 minutes at 1,260 × g. Decant the supernatant.
6. Resuspend the pellet completely in 2 mL of 1× 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 1× 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). Mix.

   Note: Each type of plant requires a different percentage of TRITON™ X-100 for proper lysis.

   - Take several µL of the cells in buffer.
   - 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 are given in the table below:

Plant	Final TRITON™ X-100 concentration
Tobacco	0.3%
Tomato	1.0%
Spinach	0.3%
Arabidopsis	0.3%

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.

Crude Preparation of Nuclei

1. Centrifuge lysate (Cell Lysis Section, Step 7) at 12,000 × g for 10 minutes in 1.5 mL tube(s).
2. Remove the supernatant.
3. Wash the pellet(s) by resuspending in 1 mL of NIBA.
4. Centrifuge for 5 minutes at 12,000 × g. If required, pool the contents of all tubes to collect the pellet.
5. At this stage, the nuclei can either be stored without further manipulations or extracted for nuclear proteins.
6. For storage:
   6.1. Remove the supernatant.
   6.2. Then resuspend the nuclei pellet in 100 µL of Nuclei PURE Storage Buffer.
   6.3. Store at −70 °C for up to three months.
7. For nuclear protein extraction, proceed to Section C.

Semi-pure Preparation of Nuclei

1. Mix the 2.3 M sucrose solution well, before use.
2. Apply the lysate (Cell Lysis Section, 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 1× NIB buffer and used as a cushion instead of the 2.3 M Sucrose.
3. Centrifuge at 12,000 × g for 10 minutes.
4. Aspirate the upper green phase and the sucrose cushion layer without disturbing the pellet of nuclei.
5. Wash the pellet(s) twice, by resuspending in 1 mL of NIBA and centrifuging for 5 minutes at 12,000 × g. If required, pool the pellets from all tubes.
6. At this stage, the nuclei can either be stored without further manipulations or extracted for nuclear proteins.
7. For storage:
   7.1. Remove the supernatant.
   7.2. Resuspend the nuclei pellet in 100 µL of Nuclei PURE Storage Buffer.
   7.3. Store at −70 °C for up to three months.
8. For nuclear protein extraction, proceed to the Nuclear Protein Extraction Section.

Highly-pure Preparation of Nuclei

1. Prepare a "gradient isolation tube":
   1.1. Dilute the Percoll® to 60% with 1× NIB (1.8 mL of Percoll® and 1.2 mL of 1× NIB).
   1.2. Put 3 mL of 2.3 M Sucrose in a 14 mL tube.
   1.3. 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 1× NIB (1.2 mL of Percoll® and 1.8 mL of 1× NIB).
   - Scaling of this Highly-pure nuclei isolation procedure can be performed using 50 mL "gradient isolation tubes" with either:
   - 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 down-scaling).
2. Carefully apply the lysate (Cell Lysis Section, Step 7) on top of the Percoll® layer.
3. Centrifuge 30 minutes at 3,200 × g. Most of the nuclei are banded at the interface between the sucrose and the Percoll® layers.
4. Gently collect the nuclei band with a Pasteur pipette.
5. Transfer into a new 14 mL tube.
6. Wash the nuclei to remove Percoll® and sucrose, by adding NIBA (2× the nuclei band volume) to the collected nuclei band.
7. Centrifuge for 5 minutes at 3,200 × g.
8. Resuspend the washed pellet in 1 mL of NIBA.
9. Transfer to a 1.5 mL tube.
10. Centrifuge 12,000 × g for 5 minutes. At this stage, the nuclei can either be stored without further manipulations or extracted for nuclear proteins.
11. For storage:
    11.1. Remove the supernatant.
    11.2. Resuspend the nuclei pellet in 100 µL of Nuclei PURE Storage Buffer.
    11.3. Store at −70 °C for up to three months.
12. For nuclear protein extraction, proceed to the Nuclear Protein Extraction Section.

Nuclear Protein Extraction

1. Prepare a Working Extraction Buffer by adding DTT to the Extraction Buffer to a final concentration of 5 mM, and also adding 1:100 (v/v) Protease Inhibitor Cocktail. The DTT and the Protease Inhibitor Cocktail should be added freshly before every extraction.
2. 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.
3. Vortex at medium-high speed for 15-30 minutes at 4 °C.
4. Centrifuge for 10 minutes at 12,000 × g.
5. Transfer the supernatant to a clean, chilled test tube.
6. For storage, snap-freeze aliquots of the supernatant in liquid nitrogen. Store at –70 °C.

Salt Removal

The nuclear proteins extracted according to the procedure are suspended in a high salt buffer. Many downstream applications, such as EMSA (electrophoretic mobility shift assay) or foot printing, require only small volumes of the nuclear protein extract. The high salt concentration is diluted by addition of the other components of the analysis.

If salts interfere with downstream research, then the salts may be removed rapidly with Sephadex™ PD-10 columns (Catalogue Number 54805). The suggested 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.

References

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