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Minute™ Total Protein Extraction Kit for Plant Tissues

Catalog number: SD-008/SN-009

Description

The Minute™ total protein extraction kit for plant tissues comprises an optimized protein extraction buffer and protein extraction filter cartridges accompanied by 2.0 ml collection tubes. Its aims to efficiently extract denatured or native proteins from various plant tissues, including leaves, seeds, soft stems, and roots. It is worth noting that the protein profiles obtained through denaturing and native cell lysis buffers differ. Therefore, depending on the specific application, one buffer may outperform the other. This kit offers both denaturing and native cell lysis buffers, allowing users to evaluate and select the most suitable option for their particular needs. By utilizing the protein extraction filter cartridges, it becomes possible to extract total plant soluble proteins from 50-200 mg of plant tissue within a short span of 5-8 minutes, while achieving a high protein yield ranging from 2-8 mg/ml.

Application

The Minute™ total protein extraction kit for plant tissues is specifically designed to swiftly extract total proteins from both fresh and frozen plant tissues. This extraction method is particularly useful for applications such as SDS-PAGE, immunoblotting, ELISA, IP, enzyme assays, and various other applications. The kit offers a highly efficient and expedited approach for obtaining soluble protein extracts from plant tissues. These extracted proteins can also serve as excellent starting materials for small-scale protein purification using column chromatography techniques.

Buffer Formulation: Proprietary

Kit components

1. 25 ml denaturing lysis buffer (SD-008)
2. 25 ml native lysis buffer (SN-009)
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. Plastic rod (2)

Shipping: This kit is shipped at ambient temperature
Storage: Store the kit at room temperature

Important Product Information

The Minute™ total protein extraction kit for plant tissues is specifically engineered to achieve rapid extraction of total proteins. The inclusion of protease inhibitors in the process is discretionary. Nevertheless, if the downstream application involves a prolonged duration or if the protein extract is intended for storage over an extended period, it is advisable to incorporate protease inhibitors into the cell lysis buffer. To accurately determine protein

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concentration, the BCA kit (Pierce) can be utilized. When investigating protein phosphorylation, it is essential to introduce phosphatase inhibitors, such as PhosStop from Roche, into buffer A prior to its application.

***** If precipitation is observed in the Denaturing Buffer at lower temperatures, it is recommended to incubate the solution at a temperature higher than 37°C until the precipitate is fully dissolved.***

Protocol:

The following procedures are intended for starting plant tissues weighing 50-100 mg, including fresh leaves, seeds, soft stems, and roots, among others. In the case of dry seeds, it is advisable to soak them in water for two days prior to use. If smaller or larger quantities of starting materials are utilized, it is recommended to adjust the amount of lysis buffer proportionally.

Denaturing Total Protein Extraction (Lysis buffer: SD-008)

1. Before starting the protein extraction process, place the protein extraction filter cartridge inside the collection tube and pre-chill it on ice.
2. **For leaves**, take 50-100 mg of fresh tissue and fold or roll the leaves to reduce their volume. Insert the prepared leaves into the filter cartridge. Using a 200/1000 µl pipette tip, punch the leaf in the filter approximately 60 times. ***Note: For tissues weighing less than 50 mg, punching is not necessary.***

   For seeds (fresh/frozen/soaked), soft stems, roots, etc., cut them into smaller pieces using a sharp blade. Place the cut pieces into the filter cartridge. Grind the tissue with a plastic rod (reusable, rinse with distilled water and dry), applying twisting force, for approximately 1 minute (around 60 times).
3. Add 50-100 µl of denaturing lysis buffer to the filter cartridge containing the tissue. Grind the tissue by using the reusable plastic rod and twisting it approximately 50-60 times.
4. Cap the filter cartridge and incubate it at room temperature for 1-2 minutes. After incubation, centrifuge the filter cartridge at top speed in a tabletop centrifuge for 2-5 minutes. Transfer the supernatant, which is the denatured total protein extract, to a fresh tube. The protein yield typically ranges from 2-6 mg/ml, depending on the type of tissues used.

****The presence of some un-lysed tissue would not affect the quality of the samples.***

Native Total Protein Extraction (Lysis buffer: SN-009)

1. Prior to initiating the protein extraction process, pre-chill the protein extraction filter cartridge inside the collection tube on ice.
2. **For leaves**, take 50-100 mg of fresh tissue and fold or roll the leaves to reduce their volume. Insert the prepared leaves into the filter cartridge. Using a 200/1000 µl pipette tip, punch the leaf in the filter repeatedly for approximately 60 times. ***Note: For tissues weighing less than 50 mg, punching is not necessary.***

   For seeds (fresh/frozen/soaked), soft stems, roots, etc., cut them into smaller pieces using a sharp blade. Place the cut pieces into the filter cartridge. Grind the tissue with a plastic rod (reusable, rinse with distilled water and dry), applying twisting force, for approximately 60 times.
3. Add 50-100 µl of native lysis buffer to the filter cartridge containing the tissue. Grind the tissue by using the reusable plastic rod and applying twisting force approximately 50-60 times.
4. Incubate the filter cartridge on ice for 5 minutes. Subsequently, centrifuge the cartridge in a microcentrifuge at top speed for 2-5 minutes at 4°C. Transfer the supernatant, which represents the native

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total protein extract, to a fresh tube. The protein yield typically ranges from 1-4 mg/ml, depending on the type of tissues used.

Troubleshooting

Problem	Solution
Low protein concentration	Increase amount of starting materials/decrease amount of lysis buffer
Low protein activity	Keep sample cold and add protease inhibitors
High protein concentration as determined by BCA assay but low protein detected in SDS-PAGE	In some plant species, the presence of polyphenolic compounds can disrupt the accuracy of protein concentration determination using the BCA assay, leading to an overestimation. To address this issue, the extracted protein can be precipitated using the Minite™ protein precipitation kit (Cat# WA-006). This kit effectively removes the interfering compounds, enabling a more precise protein quantification through the BCA assay.