Minute™ Total Protein Extraction Kit for Plant Tissues

Catalog number: SD-008/SN-009

Description

Invent Biotechnologies Minute™ total protein extraction kit for plant tissues is composed of optimized protein extraction buffer and protein extraction filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly extract denatured or native proteins from plant tissues (leaves, seeds, soft stem and roots etc.). Since protein profiles extracted by denaturing and native cell lysis buffer are not identical, for a given application one buffer might be superior to the other. This kit provides both denaturing and native cell lysis buffers for users to test and select the best one for a specific application. Due to the use of the protein extraction filter cartridges, total plant soluble proteins can be extracted from 50-200 mg plant tissue in 5-8 min with high protein yield (2-8 mg/ml).

Application

Minute™ total protein extraction kit for plant tissues is designed to rapidly extract total proteins from fresh or frozen plant tissues for applications such as SDS-PAGE, immunoblotting, ELISA, IP, enzyme assays and other applications. This kit provides a very rapid method for preparation of soluble protein extract from plant tissues. Extracted proteins can be use as a good starting material for small scale protein purification in column chromatography.

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 designed to extract total protein rapidly. The use of protease inhibitors is not necessary prior to extraction. However if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, addition of protease inhibitors to cell lysis buffer is recommended. For determination of protein concentration,

BCA kit (Pierce) can be used. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use.

If precipitate is found in Denaturing Buffer at lower temperature, incubate at >37^{0}C until the precipitate is completely dissolved.

Additional Materials Required:

Table-Top Microcentrifuge

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

Following procedures are for 50-100 mg starting plant tissues (fresh leaves, seeds and soft stem and roots etc.). For dry seeds soak them in water for two days before use. If smaller or larger amounts of starting materials are used adjust the amount of lysis buffer proportionately.

1. Prior to protein extraction pre-chill the protein extraction filter cartridge in collection tube on ice.
2. **For plant leaves**, place 50-200 mg fresh tissue in the filter by folding or rolling the leaves into smaller volume and insert into the filter cartridge. Punch the leaf in the filter repeatedly with a 200/1000 µl pipette tip for 60 times and go to step 3 (for tissues less than 50 mg punching is not necessary). **For seeds** (fresh/frozen) and soft stems cut them into smaller pieces with a sharp blade and place them in the filter cartridge; grind it with plastic rod with twisting force for 1 min (about 60 times) and go to step 3.
3. Add 50-100 µl denaturing lysis buffer to the filter. Grind the tissue with the plastic rod for 50-60 times with twisting force (Note: The plastic rod is reusable, for cleaning, rinse it thoroughly with distilled water and dry it with paper towel).
4. Cap the filter and incubate at room temperature for 1-2 min. Centrifuge at a microcentrifuge at top speed for 2-5 min. Transfer supernatant to a fresh tube (this is denatured total protein extract). The yield is typically 2-6 mg/ml depending upon type of tissues.

Important Note: 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 protein extraction pre-chill the protein extraction filter cartridge in collection tube on ice.
2. **For plant leaves**, place 50-100 mg fresh tissue in the filter by folding or rolling the leaves into smaller volume and insert into the filter cartridge. Punch the leaf in the filter repeatedly with a 200/1000 µl pipette tip for 60 times and go to step 3 (for tissues less than 50 mg punching is not necessary). For seeds (fresh/frozen) and soft stems cut them into smaller pieces with a sharp blade and place them in the filter cartridge; grind it with plastic rod with twisting force for 60 times and go to step 3.

3. Add 50-100 µl native lysis buffer to the filter. Grind the tissue with the plastic rod for 50-60 times with twisting force (Note: The plastic rod is reusable, for cleaning, rinse it thoroughly with distilled water and dry it with paper towel).
4. Incubate the filter cartridge on ice for 5 min. Centrifuge in a microcentrifuge at top speed for 2-5 min at 4°C. Transfer supernatant to a fresh tube (this is native total protein extract). The yield is typically 1-4 mg/ml depending upon type of tissues

Troubleshooting

Problem	Solution
Low protein concentration	Increase amount of starting materials/decrease amount of tissue lysis buffer
Low protein activity	Keep sample cold and add protease inhibitors