

8-min **Plant Tissue** Total Protein Extraction Kit

Cat. #: AMS.P508L (50 reactions)

Storage: The kit is shipped at ambient temperature. Store the kit at room temperature (15-25°C)

Shelf Life: 12 months

Product Description

The kit rapidly extracts denatured or native proteins from **fresh** or **frozen** plant tissues (**leaves, seeds, soft stem** and **roots** etc.). Total plant soluble proteins can be extracted from 20-200 mg plant tissue with high protein yield (2-8 mg/ml), in 5 ~ 8 minutes.

The extracted protein can be used for IP, ELISA, SDS-PAGE, immunoblottings, enzyme assays and other applications.

Product Components (This product is for research use only.)

Component	Amount	Storage
Denaturing lysis buffer	25 mL	Room temperature
Native lysis buffer	25 mL	Room temperature
protein extraction filter cartridges	50	Room temperature
collection tubes with cap	50	Room temperature
Plastic rod	4	Room temperature

Note

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 lysis buffer is recommended. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to lysis buffer prior to use.

* If precipitate is found in Denaturing Buffer, incubate at >37°C until the precipitate is completely dissolved.

Additional Materials Required

Table-Top Microcentrifuge, BCA Protein Assay Kit

Protocol:

A. Denaturing total protein extraction

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.

- 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 optional).

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.

- Add 50-100 µl **Denaturing lysis buffer** to the filter. Grind the tissue with the plastic rod for 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).
- Cap the filter and incubate at **room temperature for 1-2 minutes**. Centrifuge at a microcentrifuge at top speed for **2-5 minutes**. 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.

Note: the presence of some un-lysed tissue would not affect the quality of the samples.

B. Native Total Protein Extraction

- Prior to protein extraction pre-chill the **Native lysis buffer** and protein extraction filter cartridge in collection tube on ice.
- 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 optional).

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.

- 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).
- Incubate the filter cartridge **on ice for 5 minutes**. Centrifuge in a microcentrifuge at top speed for **2-5 minutes 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

Note: the presence of some un-lysed tissue would not affect the quality of the samples.

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 proteinase inhibitors

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