

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

**Cat. #:** 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
<a href="#">Native lysis buffer</a>	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

Remarks: This protocol is developed and validated by 101Bio's OEM partner. Spin column based protein extraction and cell. fractionation technologies were developed by 101Bio's OEM partner.

Our customers also buy:

Cat.#	Kit Name	Application	Protein Status	Minute
P501	Total protein kit	cells → total protein	denatured / native	1
P502	Total protein kit	tissues → total protein	denatured / native	1
P503	Membrane protein kit	cells / tissues → membrane and cytosol	native /detergent-free	40
P504	Nuclear protein kit	cells / tissues → nuclear & cytosol	native	6
P505	Detergent-free kit	cells → total protein	denatured / native	5
P506	Detergent-free kit	Tissues → total protein	denatured / native	5
P507	Mitochondria kit	cells / tissues → mitochondria	native/detergent-free	25
P508	Plant total protein	plant tissues → total protein	denatured / native	5
P510	Plant detergent-free	plant tissues → total protein	native	6
P511	Plant chloroplast kit	plant tissues → intact chloroplast		5
P512	Bacteria total protein	bacteria → total protein	denatured	2
P513	Nuclear envelope kit	cells → nuclear envelope	native	40
P514	Histone / DNA binding pro.	cells → histone/DNA binding pro.	denatured	10
P515	Thick cell wall microbes pro.	microbes → total protein	denatured / native	10
P516	Detergent-free thick cell wall	microbes → total protein	denatured / native	10
P517	Yeast Mitochondria	yeast → mitochondria	native	60
P518	Plant Microsomal Membrane	plant → microsomal membrane	native	60
P519	Gel slice protein recovery	gel slice → protein	denatured / native	10-20
P522	Adipose protein kit	adipose → protein	native / denatured	20
P523	Adipose fractionation kit	adipose → water soluble/insoluble	native	40
P524	Nuclei isolation kit	cells / tissues → intact nuclei	native, detergent-free	20
P525	FFPE protein kit	FFPE tissues → protein	denatured	60
P528	Endosome isolation kit	cells / tissues → endosome	denatured	20
P529	Adipose nuclei /cytosol kit	adipose tissue → nuclei & cytosol	native	30

### Protein Analysis Reagents:

P5W1	HRP Substrate Kit	Pick up your most difficult-to-detect proteins	\$99
P5W2	Antibody Enhancer	Save expensive antibodies	\$99
P5W3	WB Stripping Solution	Maximum removal of antibodies, gentle on antigen	\$99

\* For more protein analysis reagents, please visit our website: [www.101bio.com](http://www.101bio.com)