3-min **Total Protein Extraction Kit** (for Animal Cultured Cells)

Cat. #: P501S (5 rxn); P501 (20 rxn); P501L (50 rxn)

Storage: Store Native Lysis Buffer at 4°C and store Denaturing Lysis Buffer at room temperature

Shelf Life: 12 months

Product Size: 20 reactions

Product Description

3-min total protein extraction kit is the fastest total protein extraction kit for invertebrate and vertebrate cultured cells for applications such as SDS-PAGE, immunoblottings, IP, ELISA, enzyme assays and other applications.

- ✓ Optimized lysis buffer for all kinds of animal cultured cells (invertebrate and vertebrate).
- ✓ Fast – 1~8 minutes !
- ✓ Flexibility – denaturing or native lysis buffers to select
  – the extraction volume can be 20 µl to 500 µl.
- ✓ High yield – 2-8 mg/ml

This product is for research use only.

Product Components

Components	Amount			Storage
	Cat.#: **P501S**	Cat.#: **P501**	Cat.#: **P501L**	
Denaturing lysis buffer	2 mL	10 mL	25 mL	Room temperature
Native lysis buffer	2 mL	10 mL	25 mL	4°C
protein extraction filter cartridges	5	20	50	Room temperature
collection tubes with cap	5	20	50	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 cell 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.

Do not store Denaturing Lysis Buffer at 4°C, which will cause precipitation. If the Denaturing Lysis Buffer was stored at 4°C, warm it at 37°C to dissolve all precipitation completely before use.

Additional Materials Required

- 1 X PBS
- Vortexer
- Table-Top Microcentrifuge
- BCA Protein Assay Kit

Protocol of Denaturing Total Protein Extraction:

A. Non-Adherent Cells

1. Prior to protein extraction, pre-chill the protein extraction filter cartridge with collection tube on ice.

2. Harvest cells by low speed centrifugation. Wash the cells with **1 mL cold PBS** once in a 1.5 ml microcentrifuge tube and pellet the cells by centrifugation at **3,000 rpm** for **2-3 min**. Aspirate the supernatant and **leave a small amount of PBS** (about the volume of packed cells) in the tube. Vortex the tube briefly to resuspend the cells.

3. Add appropriate amounts of denaturing cell lysis buffer to the cell suspension (Table 1), vortex briefly to lyse the cells.

Note: the presence of small amount of un-lysed cells would not affect the quality of the extraction.

4. Transfer/pour the cell lysate to pre-chilled filter cartridge(s) in collection tube(s) and centrifuge in a microcentrifuge for **30 seconds** at top speed (**14,000 ~ 16,000 rpm**).

5. Immediately place the collection tube on ice. Discard the filter cartridge. The flow through is protein extract and ready for downstream applications.

Table 1 Lysis Buffer Volume for Different Packed Cell Volumes*

Packed cell volume (µL)	Denaturing lysis buffer (µL)	Equivalent cell # X 10^6
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

*For NIH3T3 and 293T cells 10 µl packed cell volume is equivalent to about10^6 cells

B. Adherent Cells

1. Prior to protein extraction pre-chill the protein extraction filter cartridge (placed in collection tube) on ice.

2. Grow adherent cells to 90-100% confluence and wash the cells once in the tissue culture plates, dishes or flasks with cold PBS, aspirate the buffer completely.

3. Add appropriate amounts of denaturing cell lysis buffer (Table 2), swirl to distribute the lysis buffer over the entire surface of tissue cultures. Scrape the lysed cells with a pipette tip or a transfer pipette and transfer the cell lysate to pre-chilled protein extraction filter cartridge(s) in collection tub(s). Centrifuge at top speed (**14,000-16,000 rpm**) in a microcentrifuge for **30 seconds**.

4. Immediately place the collection tube on ice. Discard the filter cartridge. The flow through is protein extract and ready for downstream applications.

Table 2. Amount of lysis buffer required for different amount of adherent cells

Containers	Approximate Cell#	Denaturing lysis buffer (µL)
24-well plate	0.1-0.2 Million	50
6-well plate	0.6-0.8 Million	250
25 cm^2 flask	1.5-2 Million	500

Protocol of Native Total Protein Extraction:

A. Non-Adherent Cells

1. Prior to protein extraction pre-chill Native lysis buffer and protein extraction filter cartridge with collection tube on ice.

2. Harvest the cell by low speed centrifugation. Wash the cell with cold PBS once and pellet the cells by centrifugation at **3,000 rpm** for **2-3 min**. Aspirate the supernatant and **leave a small amount of PBS** (about the volume of packed cells) in the tube. Vortex briefly to resuspend the cells.

3. Add appropriate amounts of Native lysis buffer to the cell suspension (Table 3) and vortex the tube vigorously for 15 seconds. Place the tube **on ice for 3-5 min** and vortex vigorously for 10 seconds.

4. Transfer/pour the cell lysate to pre-chilled filter cartridge, cap the tube and centrifuge in a microcentrifuge for **30 seconds** at **14,000-16,000 rpm**.

5. Immediately place the collection tube on ice and discard the filter cartridge according to your institution's waste disposal protocol. The flow through is protein extract and ready for downstream applications.

Table 3 Lysis buffer volume for different packed cell volumes*

Packed cell volume (µL)	Native lysis buffer (µL)	Equivalent cell # X 10^6
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

*For NIH3T3 and 293T cells 10 µl packed cell volume is equivalent to about10^6 cells

B. Adherent cells

1. Prior to protein extraction pre-chill Native lysis buffer and the protein extraction filter cartridge with collection tube on ice.

2. Grow adherent cells to 90-100% confluence and wash the cells twice in the tissue culture plates, dishes or flasks with PBS, aspirate the buffer completely.

3. Add appropriate amounts of Native lysis buffer (Table 4), swirl to distribute the lysis buffer over the entire surface of tissue cultures. Place the tissue culture **on ice for 5 min**. Scrape the lysed cells with a pipette tip or with a transfer pipette and transfer cell lysates to pre-chilled protein extraction filter cartridge(s), centrifuge at **14,000 to 16,000 rpm** in a microcentrifuge for **30 seconds**.

4. Immediately place the collection tube on ice and discard the filter cartridge according to your institution's waste disposal protocol. The flow through is protein extract and ready for downstream applications.

Table 4. Amount of Lysis Buffer Required for Different Amount of Adherent Cells

Containers	Approximate Cell#	Native lysis buffer (µL)
24-well plate	0.1-0.2 Million	50
6-well plate	0.6-0.8 Million	250
25 cm^2 flask	1.5-2 Million	500

Troubleshooting

Problem	Solution
The lysate is too viscous to pipette with a 200-1000 µl pipette tip	Pour the lysate into protein extraction filter cartridge
Retention of cell lysate in protein extraction filter cartridge after 30 seconds of centrifugation	Decrease amounts of starting cells/tissues or increase amount of lysis buffer
Low protein concentration Low protein band intensity at high molecular weight range (100-300 KDa)	Increase amounts of cells/tissues or decrease amount of cell lysis buffer Increase amount of lysis buffer and make sure cells/tissues are completely lysed.

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.

-- The end --