Column Tissue&Cell Protein Extraction Kit

(with Protease Inhibitor Cocktail)

C1491689

Introduction:

This kit innovatively adopts a column-based purification method to rapidly, gently, and efficiently lyse animal tissues or cells for total protein extraction. It provides both denaturing and native lysis buffers, allowing users to select the appropriate option based on downstream application requirements. The entire extraction process takes only 1–8 minutes. Thanks to the column purification technology, it can process sample-lysis buffer mixtures as small as 20μL and up to 500μL, yielding protein solutions with concentrations of 2–8 mg/mL while effectively preventing protein loss. The extracted proteins can be quantified using the BCA method (Cat. No.: R1491648/B665595).

Kit Contents and Storage conditions：

C1491689	Component	50T	Storage
C1491689A	Denaturing Lysis Buffer	25 mL	2-8℃
C1491689B	Native Lysis Buffer	25 mL	2-8℃
C1491689C	Protease Inhibitor Cocktail	500μL	-20℃
C1491689D	Purification Columns	50 units	RT.
C1491689E	Collection Tubes	50 units	RT.
C1491689F	Plastic Grinding Pestles	4 units	RT.

Key Features:

1. Simple and rapid operation: Denatured total proteins can be obtained in as little as 1 minute.
2. No protein loss: Efficiently extracts DNA-binding proteins by disrupting DNA duplexes.
3. Small sample volume, high yield: Processes mixtures as small as 20 μL, yielding protein concentrations of 2–8mg/mL.
4. Versatile applications: Includes two lysis buffers for extracting both denatured and native proteins.

Usage method:

1. Extraction of Denatured Total Protein.

1.1 Pre-chill the purification column and collection tube on ice.

1.2 Sample processing: Add protease inhibitor cocktail to the denaturing lysis buffer at a 1:100 ratio shortly before use.

1.3 Adherent cells:

- Wash cells with pre-chilled 1× PBS and aspirate the supernatant.
- Add the volume of denaturing lysis buffer specified in the appendix table to cover the culture surface, and pipette to mix.

1.4 Suspension cells:

- Collect cells by low-speed centrifugation.
- Wash with pre-chilled 1× PBS, vortex, and centrifuge at 3,000 rpm for 2–3 minutes.
- Resuspend the cell pellet in PBS equal to the pellet volume.
- Add the specified volume of denaturing lysis buffer and vortex to lyse.
  Note: Partial incomplete lysis does not affect protein extraction. If the lysate is too viscous, directly transfer it to the purification column.

1.5 Tissue samples:

- Place 15–20 mg of tissue on the purification column.
- Grind 50–60 times with a plastic pestle.
- Add 200μL denaturing lysis buffer and grind another 30–60 times.
- Adjust lysis buffer volume proportionally for larger or smaller samples.
  Note: Reusable plastic pestles should be thoroughly rinsed with distilled water and dried.

1.6 Centrifugation:

- Adherent or suspension cells: Transfer the lysate to the pre-chilled purification column and centrifuge at 14,000–16,000 rpm for 30 seconds.
- Tissue samples: Incubate the column at room temperature for 1–2 minutes, then centrifuge at 14,000–16,000 rpm for 1–2 minutes.

1.7 Immediately place the collection tube on ice and discard the purification column. Denatured total protein extraction is complete.

2. Extraction of Native Total Protein.

2.1 Pre-chill the native lysis buffer, purification column, and collection tube on ice.

2.2 Sample processing: Add protease inhibitor cocktail to the native lysis buffer at a 1:100 ratio shortly before use.

2.3 Adherent cells:

- Wash cells with pre-chilled 1× PBS and aspirate the supernatant.
- Add the specified volume of native lysis buffer and incubate on ice for 3–5 minutes. Pipette to mix.

2.4 Suspension cells:

- Collect, wash, and resuspend cells as described in section I.
- Add native lysis buffer, vortex for 15 seconds, incubate on ice for 3–5 minutes, and vortex again for 10 seconds.

2.5 Tissue samples:
Grind tissue as described in section I, using native lysis buffer.

2.6 Centrifugation:

- Adherent or suspension cells: Centrifuge at 14,000–16,000 rpm for 30 seconds.
- Tissue samples: Incubate on ice for 5 minutes (open lid), then close the lid and

centrifuge at 4°C and 14,000–16,000 rpm for 1–2 minutes.

2.7 Immediately place the collection tube on ice and discard the purification column. Native total protein extraction is complete.

Appendix: Cell Number vs. Lysis Buffer Volume：

Cell Count (×10⁶)	Lysis Buffer Volume (μL)
0.3	20
0.5	50
1	100
2	200
3	500

Matters needing attention:

1. High viscosity of the lysate is normal when using this kit.
2. For safety, wear a lab coat and disposable gloves during operation.
3. For research use only.