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ExKine™ Pro Total Protein Extraction Kit for Animal Cultured Cells and Tissues

Cat #: KTP3007 Size: 5 T/50 T

	ExKine™ Pro Total Protein Extraction Kit for Animal Cultured Cells and Tissues		
REF	**Cat:** KTP3007	LOT	**Lot number:** Refer to product label
	Protein yield: 2-8 mg/mL		**Applicable samples:** Animal cells/tissues
	Storage: Stored at room temperature for 12 months		

Assay Principle

Total protein extraction kit for animal cultured cells and tissues is a new generation of ultra fast protein extraction tool. More and more evidence shows that the most commonly used RIPA buffer may lead to random loss of protein, resulting in many difficult data that are difficult to explain. The kit uses centrifugal column extraction technology combined with optimized lysis buffer to extract total protein more quickly and effectively, making WB results more accurate. The extraction system can be as low as 20 μL by using centrifugal column to extract protein, which effectively solves the problem of small sample size. This kit is suitable for applications such as SDS-PAGE, WB and IP.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	5 T	50 T	
Denaturing cell Lysis buffer	2.5 mL	25 mL	RT
Native cell Lysis buffer	2.5 mL	25 mL	RT
Protein extraction filter cartridge	5	50	RT
Collection tube with cap	5	50	RT
Plastic rod	1	2	RT

Materials Required but Not Supplied

- Freezing centrifuge
- Centrifugal tube
- Adjustable pipette gun and gun head
- Phosphate buffer (PBS)
- Vortex mixer
- Protease inhibitor

Reagent Preparation

Denaturing cell lysis buffer: Ready to use as supplied; Store at room temperature.

Native cell lysis buffer: Ready to use as supplied; Store at room temperature.

Assay Procedure

I Denaturing total protein extraction

A. Non-Adherent cells

1. Filter cartridge in collection tube preparation: place Protein extraction filter cartridge on Collection tube with cap on ice.

2. Collect cells by low-speed centrifugation. Wash cells in cold PBS once in a 1.5 mL microcentrifuge tube and pellet the cells by centrifugation at 500 g for 2-3 min. Aspirate the supernatant and leave small amount of PBS (about the volume of cells) in the tube. Vortex the tube briefly to resuspend the cells.

3. Add appropriate amounts of Denaturing cell Lysis buffer (Table 1), and vortex briefly to lyse the cells. (The number of cells and Lysis buffer had to ensure correspondence for optimal extraction efficiency; the presence of small amount of un-lysed cells would not affect the quality of the samples)

4. Transfer the cell lysate to pre-chilled filter cartridge in collection tube and centrifuge at 14,000 g for 30 s.

5. Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol. The cell lysate is now ready for downstream applications.

Table 1 Lysis buffer volume for different cell volumes

Cell volume（μL）	Lysis buffer（μL）	Number of cells (×10^6)
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

B. Adherent cells

1. Filter cartridge in collection tube preparation: place Protein extraction filter cartridge on Collection tube with cap on ice.

2. Add the cold PBS directly into the culture plate, culture dish or culture flask, clean the adherent cells, and discard the supernatant.

3. Add appropriate amounts of denaturing cell lysis buffer (Table 2) to the surface of the whole containers, blow several times, then transfer the cell lysate to pre-chilled filter cartridge in collection tube and centrifuge at 14,000 g for 30 s. (if the extraction concentration is poor, reduce the volume of cell Lysis buffer)

4. Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol. The cell lysate is now ready for downstream applications.

Table 2 Lysis buffer volume for different adherent cell volumes

Containers	Lysis buffer（μL）	Number of cells (×10^6)
24-well plate	50	0.1-0.2
6-well plate	200	0.6-0.8
25 cm^2 flask	500	1.5-2

C. Tissues

1. Filter cartridge in collection tube preparation: place Protein extraction filter cartridge on Collection tube with cap on ice.

2. Place 15-20 mg fresh/frozen tissue in the filter cartridge. Grind the tissue with a plastic rod for 50-60 time with twisting force,

add 200 μL denaturing cell lysis buffer to the filter and continue to grind for 30-60 times. (The amount of tissue should not be excessive, and there is no need for excessive grinding. The Lysis buffer can be added twice to obtain the best effect. If the initial amount of tissue is large or small, adjust the amount of Lysis buffer proportionally)

3．Cap the filter cartridge and incubate at room temperature for 1-2 min. Centrifuge at 14,000 g for 1-2 min. The supernatant of Collection tube with cap contains denatured total protein extract.

II Native total protein extraction

A. Non-Adherent cells

1．Filter cartridge in collection tube preparation: place Protein extraction filter cartridge on Collection tube with cap on ice.

2．Collect cells by low-speed centrifugation. Wash cells in cold PBS once in a 1.5 mL microcentrifuge tube and pellet the cells by centrifugation at 500 g for 2-3 min. Aspirate the supernatant and leave small amount of PBS (about the volume of cells) in the tube. Vortex the tube briefly to resuspend the cells.

3．Add appropriate amounts of Native cell Lysis buffer (Table 3), and vortex briefly to lyse the cells. (The number of cells and Lysis buffer had to ensure correspondence for optimal extraction efficiency; the presence of small amount of un-lysed cells would not affect the quality of the samples)

4．Transfer the cell lysate to pre-chilled filter cartridge in collection tube and centrifuge at 14,000 g, 4℃ for 30 s.

5．Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol. The cell lysate is now ready for downstream applications.

Table 3 Lysis buffer volume for different cell volumes

Cell volume (μL)	Lysis buffer (μL)	Number of cells (×10^6)
3	20	0.3
5	50	0.5
10	100	1
20	200	2
40	500	3

B. Adherent cells

1．Filter cartridge in collection tube preparation: place Protein extraction filter cartridge on Collection tube with cap on ice.

2．Add the cold PBS directly into the culture plate, culture dish or culture flask, clean the adherent cells, and discard the supernatant.

3．Add appropriate amounts of native cell lysis buffer (Table 4) to the surface of the whole containers, blow several times, then transfer the cell lysate to pre-chilled filter cartridge in collection tube and centrifuge at 14,000 g, 4℃ for 30 s. (if the extraction concentration is poor, reduce the volume of cell Lysis buffer)

4．Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol. The cell lysate is now ready for downstream applications.

Table 4 Lysis buffer volume for different adherent cell volumes

Containers	Lysis buffer（μL）	Number of cells (×10^6)
24-well plate	50	0.1-0.2
6-well plate	200	0.6-0.8
25 cm^2 flask	500	1.5-2

C. Tissues

1．Filter cartridge in collection tube preparation: place Protein extraction filter cartridge on Collection tube with cap on ice.

2．Place 15-20 mg fresh/frozen tissue in the filter cartridge. Grind the tissue with a plastic rod for 50-60 time with twisting force, add 200 μL native cell lysis buffer to the filter and continue to grind for 30-60 times. (The amount of tissue should not be excessive, and there is no need for excessive grinding. The Lysis buffer can be added twice to obtain the best effect. If the initial amount of

tissue is large or small, adjust the amount of Lysis buffer proportionally)

3. Open the cap and incubate for 5 min on ice. Centrifuge at 14,000 g, 4℃ for 1-2 min. The supernatant of Collection tube with cap contains native total protein extract.

Precaution

1. Protease inhibitors are not necessarily added, but if downstream experiments take longer or are preserved for longer periods after protein extraction, it is advisable to add protease inhibitors. The BCA kit is recommended for protein concentration determination. To study protein phosphorylation, phosphatase inhibitors should be added to the cell lysis buffer before use.
2. Before WB, still mix the sample and loading buffer to boiling.
3. The plastic rod is reusable, rinse thoroughly with distilled water, and dry it with paper towel.
4. If the lysate is too viscous to pipette with a 200-1000 μL pipette tip, pour the cell lysate into the filter cartridge or cut the tip off.
5. If the cell lysis buffer also remains in the centrifuge tube after centrifugation for 30 s, reduce the number of starting cells/ tissues or increase the volume of cell lysis buffer.
6. If the protein concentration is low, increase the number of starting cells / tissues or reduce the volume of cell lysis buffer.
7. If weak protein bands in the high molecular weight range (100-300 kDa), increase the volume of cell lysis buffer to ensure adequate lysis.

FAQ

1. Can the tissue be treated with a homogenizer to extract total protein with this kit?

A: It is recommended to use the grinding rod provided to grind the sample directly in the centrifugal pipe string, which is easy to operate and saves time and effort. For tissue samples that are not easy to grind, a homogenizer can be used for processing, and pay attention to keeping low temperature during operation.

2. Can the kit extract total protein from bone tissue and adipose tissue?

A: It can be applied to the extraction of cartilage tissue, hard tissue such as bone and teeth and adipose tissue have not been verified, so it is not recommended to use this kit for the extraction of total protein.

3. Why is there occasionally a small amount of flocculent in the collection tube after extraction and centrifugation?

A: After animal tissue or cell samples are cracked, a small amount of grease substances enter the collection tube through the filter element and condense into solid grease at low temperature to form a white flocculent. It is recommended to use the clarified liquid in the tube for downstream experiments.

Recommended Products

Catalog No.	Product Name
KTD3001	Protein Quantification Kit (BCA Assay)
BMP1001	Protease Inhibitor Cocktail (100×)
BMU103	SuperKine™ Enhanced Antibody Dilution Buffer
BMU102	SuperKine™ West Femto Maximum Sensitivity Substrate
BMU101	SuperKine™ West Pico PLUS Chemiluminescent Substrate

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.