Minute™ Total Protein Extraction Kit for Mass Spectrometry

Catalog Number: MS-026

Description

Mass spectrometry has become a popular method for analyzing and characterizing proteins. Traditional total protein extraction methods, such as RIPA-based reagents, can alter endogenous baselines due to nonsystematic protein loss. Some chemicals in RIPA may not be compatible with mass spectrometry and other downstream applications. This kit extracts total proteins quickly using a specially formulated lysis buffer and proprietary spin column. The extracted proteins are compatible with trypsin digestion followed by MS analysis, iTRAQ, biotin labeling, etc.… Due to the use of filter cartridges, the extraction volume can be as low as 20 µl – a helpful feature when starting material is a limiting factor. The typical yield is 2-8 mg/ml.

Kit components

1. 25 ml Lysis Buffer
2. 50 protein extraction filter cartridges
3. 50 collection tubes with cap
4. Plastic rods (2)

Shipping: Ship at ambient temperature
Storage: Store at room temperature

Important Product Information

This kit is designed to extract total protein for MS analysis. The use of protease inhibitors is optional. However, if the downstream application takes significant time or the extracted protein will be stored for a more extended period, the addition of protease inhibitors to the lysis buffer is recommended. A BCA kit (Pierce) is recommended for determining protein concentration. For protein phosphorylation studies, **phosphatase inhibitors** (e.g., PhosStop from Roche) should be added to the lysis buffer use.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge
BCA Protein Assay Kit (Pierce, Cat #. 23227)
Cold Acetone

Protocols

- ### Total Protein Extraction for Cultured Cells

A. Non-Adherent Cells

1. Pre-chill the filter cartridge in a collection tube on ice.

2. Harvest cells by low-speed centrifugation. Wash the cells in cold PBS once in a 1.5 ml microcentrifuge tube and pellet the cells by centrifugation at 500 X g for 2-3 min. Aspirate the supernatant and leave a small amount (about the volume of packed cells) in the tube, vortex to resuspend the cells.
3. Add cell lysis buffer to the cell suspension (Table 1) and vortex briefly to lyse the cells.
4. Transfer/pour the cell lysate into a pre-chilled filter cartridge in a collection tube and centrifuge for 30 seconds at 16,000 X g.
5. Immediately place the collection tube on ice. Discard the filter cartridge.
6. Add cold acetone (1 part of protein extract + 6 parts of acetone stored at -20ºC). Mix well and store at -20ºC for 1-12 hrs (overnight incubation is recommended if protein concentration is less than 1mg/ml).
7. Centrifuge at 14,000 X g for 10-15 min at 4ºC. Remove the supernatant and let air dry. The pellet (total protein) can be dissolved in a buffer depending upon specific downstream applications (see Tech Notes below)

Table 1. Lysis Buffer Volume for Different Packed Cell Volumes*

Packed cell volume (µl)	lysis buffer (µl)	Equivalent cell # X 10^7
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 about10^7 cells

B. Adherent cells

1. Pre-chill the filter cartridge in a collection tube on ice.
2. Wash the cells (>90% confluence) once with cold PBS, and aspirate the buffer completely.
3. Add lysis buffer (Table 2), and swirl to distribute the lysis buffer evenly. Scrape the lysed cells and transfer the cell lysate to a pre-chilled filter cartridge. Centrifuge at top speed (14,000-16.000 X g) for 30 seconds. Discard the filter and place the collection tube on ice.
4. Add cold acetone (1 part of protein extract + 6 parts of acetone stored at -20ºC). Mix well and store at -20ºC for 1-12 hrs (overnight incubation is recommended if protein concentration is less than 1mg/ml).
5. Centrifuge at 14,000 X g for 10-15 min at 4ºC. Remove the supernatant and let air dry. The pellet (total protein) can be dissolved in a buffer depending upon specific downstream applications (see Tech Notes below)

Table 2 Amounts of lysis buffer required for different amount of adherent cells

Containers	Approximate Cell#	Lysis buffer(µl)
24-well plate	0.1-0.2 Million	50
6-well plate	0.6-0.8 Million	200
25 cm^2 flask	1.5-2 Million	500

- **Total Protein Extraction from Animal Tissues**

The following procedure is for 15-20 mg of tissues. The amount of lysis buffer can be adjusted proportionately according to the actual tissue amount.

1. Pre-chill the filter cartridges in collection tubes on ice.
2. Place 15-20 mg of fresh/frozen tissue in the filter cartridge. Grind the tissue using twisting force 50-60 times with a plastic rod, add 200 µl cell lysis buffer and continue to grind 30-60 times.
   Note: The plastic rod is reusable. Rinse it thoroughly with distilled water and dry it with a paper towel.
3. Incubate at room temperature with the cap open for 2-3 min. Centrifuge at top speed for 1 min. Discard the filters and place the collection tubes containing the protein extract on ice.
4. Add 6 X of cold acetone (1 part of protein extract + 6 parts of acetone stored at -20°C). Mix well and store at -20°C for 1-12 hrs.
5. Centrifuge at 14,000 X g for 10-15 min at 4°C. Remove the supernatant and let air dry. The pellet (total protein) can be dissolved in a buffer depending upon specific downstream applications (see Tech Notes below)

Tech Notes:

Acetone precipitated proteins can be dissolved in different buffers for different applications. Followings are some examples:

A. **Trypsin digestion:** Resuspend the dried pellet from acetone precipitate in 50 mM sodium bicarbonate with 0.1% SDS (ph.8.0). Determine protein concentration and dilute protein (5-10 folds) to the desired concentration with 50 mM Na-bicarbonate. In most cases, extracted protein concentration with this kit is high enough for direct trypsin digestion. Check protein concentration by BCA assay and take the desired amount of protein for trypsin digestion.
B. **iTRAQ labeling:** Resuspend the dried pellet in 6M Urea in 50mM triethylammonium bicarbonate (TEAB). Dilute the suspension with 50mM TEAB so that the urea concentration is less than 1M before trypsin digestion.
C. **Biotin labeling:** Resuspend dried pellet in 0.1% Tween-20, 50 mM sodium bicarbonate, 2% lauryl maltoside. (ph.8-8.5).