Minute™ Total Protein Extraction Kit for Mass Spectrometry

Catalog Number: MS-026

Description

Mass spectrometry has become a popular method for analysis and characterization of proteins. Traditional total protein extraction method such as RIPA buffer-based protein extraction suffers the disadvantages of incomplete protein extraction. Some components in RIPA buffer may not compatible with certain downstream applications. Minute™ total protein extraction kit for mass spectrometry is specifically developed to overcome these short comings. This kit can extract total protein from animal cultured cells/tissues efficiently and rapidly without bias. The extracted proteins are compatible with trypsin digestion, iTRAQ labeling and biotin-labeling. Due to the use of the protein extraction filter cartridges, the extraction volume can be as small as 20 µl and as large as 500 µl. This unique feature is very useful in situations where available starting material is a limiting factor. Total proteins can be extracted rapidly with high yield (2-8 mg/ml).

Application

This kit effectively extracts total proteins for applications such as trypsin digestion followed by mass spectrometry analysis, iTRAQ, biotin labeling and other applications.

Buffer Formulation: Proprietary

Kit components(50T):

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

Shipping: This kit is shipped at ambient temperature
Storage: Store the kit at room temperature

Important Product Information

This kit designed to extract total protein rapidly. The use of protease inhibitors is optional 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 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.

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. 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 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 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 cell lysis buffer to the cell suspension (Table 1), vortex briefly to lyse the cells.
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 X g).
5. Immediately place the collection tube on ice. Discard the filter cartridge according to your institution's waste disposal protocol.
6. Add 6 X of cold acetone (1 part of protein extract + 6 parts of acetone store at -20^{o}C). Mix well and store at -20^{o}C for at least 1h to overnight (overnight incubation is recommended for proteins <1mg/ml).
7. After incubation, centrifuge in a table top microfuge at top speed (14,000-16,000 X g) for 10-15 min at 4^{o}C. Remove the supernatant and let air dry. The pellet contains total extracted proteins. The pellet can be dissolved in different buffers depending upon specific downstream applications (see below)

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

Packed cell volume (µl)	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 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 X g) in a microcentrifuge for 30 seconds. Discard the filter and place the collection tube on ice.
4. Add 6 X of cold acetone (1 part of protein extract + 6 parts of acetone store at -20°C). Mix well and store at -20°C for at least 1h to overnight (overnight incubation is recommended for proteins <1mg/ml).
5. After incubation, centrifuge in a table top microfuge at top speed (14,000-16,000 X g) for 10-15 min at 4°C. Remove the supernatant and let air dry. The pellet contains total extracted proteins. The pellet can be dissolved in different buffers depending upon specific downstream applications (see 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

Following procedures are for 15-20 mg starting animal tissues. If smaller or larger amounts of starting materials are used adjust the amount of cell lysis buffer proportionately.

1. Prior to protein extraction pre-chill the protein extraction filter cartridge in collection tube on ice.
2. Place 15-20 mg fresh/frozen tissue in the filter. Grind the tissue with a plastic rod for 50-60 time with twisting force, add 200 µl cell lysis buffer to the filter and continue to grind for 30-60 times. Note: The plastic rod is reusable. For cleaning, rinse it thoroughly with distilled water and dry it with paper towel.
3. Incubate at room temperature with cap open for 2-3 min. Centrifuge at a microcentrifuge at top speed (14,000-16,000 X g) for 1 min. Discard the filter and place the collection tube with protein extract on ice.
4. Add 6 X of cold acetone (1 part of protein extract + 6 parts of acetone store at -20°C). Mix well and store at -20°C for at least 1h to overnight.

5. After incubation, centrifuge in a table top microfuge at top speed (14,000-16,000 X g) for 10-15 min at 4°C. Remove the supernatant and let air dry. The pellet contains total extracted proteins. The pellet can be dissolved in different buffers depending upon specific downstream applications (see below).

   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 to desired concentration with 50 mM Na-bicarbonate. In most cases extracted protein concentration with this kit is high enough for direct trypsin digestion without acetone precipitation. The extract can be diluted 5-10 fold with 50 mM sodium bicarbonate. After dilution check protein concentration by BCA assay and take desired amount for trypsin digestion.
   B. **iTRAQ labeling:** Resuspend the dried pellet in 6M Urea in 50mM triethylammonium bicarbonate (TEAB). Dilute the extracts in 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).