Minute™ Total Protein Extraction Kit for Animal Cultured Cells and Tissues

Catalog Number: SD-001/SN-002

Description

Minute™ total protein extraction kit for animal cultured cells and tissues is the most advanced next generation protein extraction tool for super-fast protein extraction without altered protein profile usually associated with solution based procedures. More and more evidences have shown that the most commonly used RIPA buffer can cause unpredictable protein loss, resulting in questionable data interpretation. This problem is fully resolved by the patented spin column based technologies. Coupled with much stronger lysis buffers, proteins can be extracted more efficiently. Due to the use of the proprietary protein extraction filter cartridges, the extraction volume can be as low as 20 µl – a very useful feature in situations where starting material is a limiting factor. This kit provides both denaturing and native cell lysis buffers so users can select according to specific applications. Total proteins can be extracted from cultured cells/tissues in 1-8 min with high yield (2-8 mg/ml).

Selected references related to protein loss by RIPA buffer:

1. Bai,B., and Laiho, M. (2012) Proteomics. 12:3044-3048
2. Mukhopadhyay, C. et al. (2016) PNAS 5:8228-8237
3. Li, Q. (2016) Biotechniques. 61:327
4. Ngoka, L. CM. (2008) Proteome Science. 6:30

Application

Minute™ total protein extraction kit is designed to rapidly extract total proteins from invertebrate and vertebrate cultured cells and tissues for applications such as SDS-PAGE, immunoblotting, IP, ELISA, enzyme assays and other applications. This kit provides the most rapid method currently available for preparation of whole cell protein extract. Extracted proteins can also be used as a good starting material for small scale protein purification in column chromatography.

Buffer Formulation: Proprietary

Kit components

1. 25 ml denaturing cell lysis buffer (SD-001)
2. 25 ml Native cell lysis buffer (SN-002)
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. Plastic rods (2)

NOTE:* ***Cell lysis buffers listed above do not contain any reducing agents and primary amine

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

Important Product Information

The Minute™ total protein extraction kits are designed to extract total protein rapidly. 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.

*****If precipitate is found in Denaturing Buffer at lower temperature, incubate at >37^{0}C until the precipitate is completely dissolved.***

Additional Materials Required

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

Protocols:

Total Protein Extraction for Cultured Cells

- **Denaturing Total Protein Extraction (SD-001)**

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.

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

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 (14,000-1,6000 X g).
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 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, this is a general reference volume, the actual amount of lysis buffer can be more or less), Scrape the lysed cells with a pipette tip or a transfer pipette and pipette up and down repeatedly to lyse the cells. 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.
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 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

- **Native Total Protein Extraction (SN-002)**

A. Non-Adherent Cells

1. Prior to protein extraction pre-chill native cell lysis buffer (SN-002) and protein extraction filter cartridge with collection tube on ice.
2. Harvest the cell by low speed centrifugation. Wash the cell in cold PBS once 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 briefly to resuspend the cells.
3. Add appropriate amounts of 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 X g.
5. Immediately place the collection tube on ice and 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 packed cell volumes*

Packed cell volume (µl)	lysis buffer (µl)	Equivalent cell # x 10^6
3	25	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 cell lysis buffer (SN-002) 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 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 X g 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 cell lysate is now ready for downstream applications.

Table 4 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	250
25 cm^2 flask	1.5-2 Million	500

Total Protein Extraction for Animal Tissues

- **Denaturing Total Protein Extraction (SD-001)**

Following procedures are for 15-20 mg starting animal tissues. If smaller or larger amount of starting material is 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 denaturing 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. Cap the filter and incubate at room temperature for 1-2 min. Centrifuge at a microcentrifuge at top speed for 1-2 min. The supernatant of flow through contains denatured total protein extract.

Important Note: the presence of small amount of un-lysed tissue would not affect the quality of the samples

- **Native Total Protein Extraction (SN-002)**

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 native lysis buffer (SN-002) 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. After tissue grinding, incubate the tube on ice with cap opening for 5 min. Cap the filter and centrifuge in a microcentrifuge at top speed for 1-2 min at 4°C. The supernatant of flow through contains native total protein extract.

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.