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 tool for super-fast protein extraction without altered protein profile usually associated with solution-based procedures. More and more evidence has shown that the most commonly used RIPA buffer can cause unpredictable protein loss, resulting in questionable data interpretation. This problem is fully resolved using the patented spin column-based technologies. Coupled with stronger lysis buffers, the spin column makes protein extraction super easy and efficient. The extraction volume can be as low as 20 µl – a helpful feature when starting material is a limiting factor. This kit provides 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, and enzyme assays. This kit provides the most rapid method currently available for the preparation of the total protein extracts. Extracted proteins can also be used as a good starting material for small-scale protein purification in column chromatography.

Kit components(50 Preps):

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)

Kit components(4 Preps):

1. 2.0 ml denaturing cell lysis buffer (SD-001)
2. 2.0 ml Native cell lysis buffer (SN-002)
3. 4 protein extraction filter cartridges
4. 4 collection tubes with cap
5. Plastic rods (1)

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 quickly extract total proteins. 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 before 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. Pre-chill the filter cartridges (placed in collection tubes) on ice.
2. Harvest cells by low-speed centrifugation. Wash the cells with 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 of PBS (about the volume of packed cells) in the tube. Vortex briefly to resuspend the cells.
3. Add appropriate amounts of cell lysis buffer to the cell suspension (Table 1) and vortex briefly to lyse the cells. ***Note: 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(s) in collection tube(s) and centrifuge for 30 seconds at top speed (14,000-1,6000 X g).
5. Discard the filter cartridge. The sample 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 10^7
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***
Note: this is a general reference, the actual amount of lysis buffer can be more or less

B. Adherent cells

1. Pre-chill the filter cartridges (placed in collection tubes) on ice.
2. Grow cells to 90-100% confluence and wash the cells with cold PBS. Aspirate the buffer completely.
3. Add appropriate amounts of cell lysis buffer (Table 2). Add appropriate amounts of cell lysis buffer (Table 2). Pipette repeatedly to lyse the cells. Transfer the cell lysate to a filter cartridge in a collection tube. Centrifuge at top speed (14,000-16,000 X g) for 30 seconds.
4. Discard the filter cartridge. The sample 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

Note: this is a general reference, the actual amount of lysis buffer can be more or less

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

A. Non-Adherent Cells

1. Pre-chill native cell lysis buffer (SN-002) and filter cartridges with collection tubes on ice.
2. Harvest cells by low-speed centrifugation. Wash the cells with 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 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 vigorously for 15 seconds. Incubate on ice for 3-5 min and vortex for 10 seconds.
4. Transfer the cell lysate into a pre-chilled filter cartridge, cap, and centrifuge for 30 seconds at 14,000-16,000 X g.
5. Discard the filter cartridge. The sample 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 10^7
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 about10^7 cells

Note: this is a general reference, the actual amount of lysis buffer can be more or less

B. Adherent cells

1. Pre-chill native cell lysis buffer (SN-002) and the filter cartridges with collection tubes on ice.
2. Grow cells to 90-100% confluence and wash the cells twice with cold PBS. Aspirate the buffer completely.
3. Add appropriate amounts of lysis buffer (Table 4), and swirl to cover the entire surface. Place on ice for 5 min. Pipette repeatedly to lyse the cells. Transfer the cell lysate to a filter cartridge (placed in a collection tube), and centrifuge at top speed (14,000-16,000 X g) for 30 seconds.
4. Discard the filter cartridge. The sample 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

Note: this is a general reference, the actual amount of lysis buffer can be more or less

Total Protein Extraction for Animal Tissues

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

Following procedures are for 15-20 mg starting animal tissues. For different amount, adjust the amount of cell lysis buffer proportionately.

1. Pre-chill the filter cartridges with collection tubes on ice.
2. Place 15-20 mg of fresh/frozen tissue in the cartridge. Grind the tissue with a plastic rod 50-60 times with the twisting force, add 200 µl denaturing cell lysis buffer (SD-001) to the filter and continue to grind 30-60 times.
   Note: The plastic rod is reusable. Rinse with distilled water and dry it with a paper towel.
3. Cap the cartridge and incubate at room temperature for 1-2 min. Centrifuge at top speed for 1-2 min.
4. Discard the filter cartridge. The sample is now ready for downstream applications.

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. Pre-chill the filter cartridges with collection tubes on ice.
2. Place 15-20 mg of fresh/frozen tissue in the cartridge. Grind the tissue with a plastic rod 50-60 times with the twisting force, add 200 µl native lysis buffer (SN-002) to the filter and continue to grind 30-60 times.
   Note: The plastic rod is reusable. Rinse with distilled water and dry it with a paper towel.
3. Incubate on ice with the cap opened for 5 min. Cap the cartridge and centrifuge at top speed for 1-2 min at 4°C.
4. Discard the filter cartridge. The sample is now ready for downstream applications.

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 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.

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