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

Cat #: SD-001/SN-002

Description

The Minute™ kit is a next-generation, spin column–based system designed for rapid and efficient total protein extraction from animal cultured cells and tissues. Unlike conventional solution-based methods, such as RIPA buffer, which often result in protein loss and altered profiles, this patented technology preserves protein integrity and yields reproducible results.

With enhanced lysis buffers and a streamlined protocol, proteins can be extracted in just 1–5 minutes at concentrations ranging from 1–8 mg/ml. The system supports both denaturing and native conditions, allowing users to choose based on downstream applications. Minimal extraction volume (as low as 20 µl) makes it ideal when starting material is limited.

Application

The Minute™ Total Protein Extraction Kit enables rapid and efficient isolation of total proteins from both invertebrate and vertebrate cultured cells and tissues. It is optimized for common downstream applications including SDS-PAGE, Western blotting, immunoprecipitation (IP), ELISA, and enzyme activity assays.

This kit offers one of the fastest protocols available for total protein preparation and can be readily adapted for high-throughput workflows. The extracted proteins are also suitable as starting material for small-scale purification using 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

Additional Guidelines for Optimal Use

The Minute™ Total Protein Extraction Kits are engineered for rapid and efficient protein extraction. The inclusion of protease inhibitors in the lysis buffer is optional; however, their use is recommended if the extracted proteins will be stored or if downstream applications are time-consuming, to prevent proteolytic degradation.

For accurate protein quantification, the BCA Protein Assay Kit (Pierce) is recommended.

For phosphorylation studies, phosphatase inhibitors—such as PhosSTOP (Roche)—should be added to the lysis buffer prior to use to preserve phosphorylation states.

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

1. Place a filter cartridge into a collection tube and keep it on ice.
2. Harvest cells by low-speed centrifugation. Wash the cell pellet once with cold PBS in a 1.5 ml microcentrifuge tube.
3. Pellet cells by centrifugation at 500-600 X g for 3 min. Discard the supernatant.
4. Add 20-500 µl of lysis buffer (SD-001 or SN-002) to the cell pellet. Vortex for 10–20 seconds to fully lyse the cells. ***Refer to Tables 1–4 for recommended lysis buffer-to-cell ratios.***
5. Transfer the lysate to the filter cartridge in the collection tube. Centrifuge at 14,000–16,000 × *g* for 30 seconds using a tabletop centrifuge.
6. Discard the filter cartridge. The filtrate in the collection tube contains the extracted total protein, ready for downstream applications.

Total Protein Extraction for Animal Tissues

1. Place 1–20 mg of fresh or frozen tissue into the filter cartridge positioned in the collection tube. Using a plastic rod, grind the tissue with a twisting motion 20–30 times.
2. Add 50–200 µl of lysis buffer (SD-001 for denaturing or SN-002 for native extraction) directly into the filter cartridge. Continue grinding for another 10–20 strokes to complete tissue homogenization.
   Note: The plastic rod is reusable. Rinse thoroughly with distilled water and dry with a clean paper towel.
3. Cap the filter cartridge and centrifuge at maximum speed (14,000–16,000 X *g*) for 30 seconds to 1 minute using a tabletop centrifuge.
4. Discard the filter cartridge. The filtrate in the collection tube contains the extracted total protein, ready for downstream applications.

High-Throughput Applications

The filter cartridges, in combination with lysis buffers (SD-001 or SN-002), are well-suited for high-throughput applications, enabling rapid and consistent extraction of total protein from multiple animal tissue samples or cultured cells. For optimal performance in high-throughput workflows, a sample mixer or vortexer equipped with an attachment capable of holding multiple 2 mL Eppendorf tubes, and operating at a minimum speed of 2,500 rpm, is required.

1. Place 5–30 mg of fresh or frozen tissue into the filter cartridges seated in collection tubes.
2. Add 100–300 µl of lysis buffer (SD-001 for denaturing or SN-002 for native conditions) into each filter cartridge. Cap the filters securely and placed in a multisampling adaptor.
3. Vortex the sealed filter cartridges in their collection tubes at 2,000–2,500 rpm for 3–5 minutes at room temperature.

4. After vortexing, transfer all samples—along with the mixing attachment—into a compatible rotor adapter (e.g., a 96-well plate adapter) and centrifuge at maximum speed for 1–2 minutes. Alternatively, remove the filter cartridges along with the collection tubes and centrifuge them in a tabletop centrifuge at top speed for 30 seconds to 1 minute.
5. The filtrate collected in the tube is the total protein extract, ready for downstream applications. Protein yield typically ranges from 1–5 mg/ml.

Note: a). For cultured cell samples, please refer to Tables 1–4 for buffer volumes and handling. Vortexing (Step 3) can generally be omitted.
b). While increased vortex speed and duration may enhance protein yield, do not exceed 28,000 rpm or vortex for longer than 10 minutes, as this may compromise protein integrity.

Recommendations for Protein Extraction from Cultured Cells

-Total Protein Extraction Using Denaturing Lysis Buffer (SD-001)

A. **For Cells in Suspension**. Harvest cells by centrifugation at 500-600 X g for 3 min. Wash cells with 1 X cold PBS and estimate wet cell pellet volume.

Table 1. Lysis Buffer Volume Recommended 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

B. **For Adherent cells.** Wash cells with 1 X PBS and lyse cells in the culture container by pipetting up and down for 10-20 times.

Table 2. Amounts of lysis buffer recommended for different number 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

-Total Protein Extraction Using Native Lysis buffer (SN-002)

A. For Cells in Suspension. Harvest cells by centrifugation at 500-600 X g for 3 min. Wash cells with 1 X PBS and estimate wet volume.

Table 3. Lysis buffer volume recommended 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

B. For Adherent cells. Wash cells with 1 X PBS and lyse cells in the culture container by pipetting up and down for 10-20 times

Table 4. Amounts of Lysis Buffer recommended 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

Tech Notes:

1. The protocols can be performed at room temperature.
2. The filter cartridge may accommodate more starting material than recommended, as long as it does not become clogged after centrifugation.
3. The cells can be washed with 0.5 to 1 ml cold PBS 1-2 times.
4. Denaturing buffer usually yields more protein than native buffer because of high cell lysis efficiency.
5. If very small amount of tissue is used (<5 mg), incubation at room temperature for 5 min after plastic rod homogenization may increase the yield.
6. Extracted protein may be stored at -80°C for long term storage.

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.