

***Minute*™ Total Protein Extraction Kit (For Animal Cultured Cells and Tissues)**

Catalog Number: SD-001/SN-002

Description

Invent Biotechnologies *Minute*™ total protein extraction kit for animal cultured cells and tissues is composed of optimized cell lysis buffer and protein extraction filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly extract total proteins from animal cells and tissues (invertebrate and vertebrate) for protein analysis and further purification. Since protein profiles extracted by denaturing and native cell lysis buffer are not identical, for a given application one lysis buffer might be superior to the other. This kit provides both denaturing and native cell lysis buffers for users to test and select the best one for a specific application. 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 ul. This unique feature is very useful in situations where available starting material is a limiting factor. Total proteins can be extracted from cultured cells/tissues in 1-8 min with high yield (2-8 mg/ml).

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, immunoblottings, 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 use 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 (4)

Storage

Store native cell lysis buffer (SN-002) at 4°C and rest of 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.

Additional Materials Required

1 X PBS

Vortexer

Table-Top Microcentrifuge

BCA Protein Assay Kit (Pierce, Cat #. 23227)

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 3000 rpm 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 (14,000-16,000 rpm).
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 about 10⁶ 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 rpm) 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(ul)
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 3000 rpm 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 rpm.
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 about 10⁶ 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 rpm 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. For insect such as *Drosophila* use 15- 20 larva, pupa or adult flies/sample. 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 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	Increase amounts of cells/tissues or decrease amount of cell lysis buffer
Low protein band intensity at high molecular weight range (100-300 KDa)	Increase amount of lysis buffer and make sure cells/tissues are completely lysed.