Minute™ Detergent-Free Protein Extraction Kit for Animal Cultured Cells and Tissues

Cat#: SN-006

Description

Invent Biotechnologies Minute™ detergent-free total protein extraction kit 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 cultured cells (insect /mammalian/other cultured cells) and animal tissues (invertebrate and vertebrate). The protein extraction buffers do not contain any detergent and EDTA. 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. Detergent-free total proteins can be extracted from cultured cells/tissues in less than 5 min with high yield (1-5 mg/ml).

Application

Minute™ detergent-free total protein extraction kit is designed to rapidly extract total proteins from invertebrate and vertebrate cultured cells/tussues for applications such as proteomics (LC/MS), IP, ELISA, 2D-gel analysis, isoelectric focusing, SDS-PAGE, immunoblottings, and other applications. This kit provides a very rapid method for preparation of high concentration of whole cell extract. Because of high yield of protein extract and low salt concentration, the protein extract can be used directly for downstream applications without desalting and protein concentration.

Buffer Formulation: Proprietary

Kit components

1. 15 ml buffer A
2. 15 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. Plastic rods (4)

Storage: Store the kit at 4^{o}C

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 buffer A is

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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 buffer A prior to use.

Additional Materials Required

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

Protein Extraction Procedures for Cultured cells

A. Non-Adherent Cells

1. Prior to protein extraction, pre-chill buffers and the protein extraction filter cartridge with collection tube on ice.
2. Harvest cells by low speed centrifugation. Wash the cells in a microcentrifuge tube with 1 ml cold PBS 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 buffer A to the cell suspension (Table 1), vortex 10-20 seconds to lyse the cells. Add equal volume of buffer B to the tube and mix well by vortexing briefly.

   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 in collection tube and centrifuge in a microcentrifuge for 30 seconds to one min at top speed (14,000-16,000 rpm). Transfer the supernatant of flow through to a pre-chilled microcentrifuge tube. The protein extract is now ready for downstream applications.

Table 1 Buffers Required for Different Packed Cell Volumes*

Packed cell volume (µl)	Buffer A (µl)	Buffer B (µl)
5	20	20
10	50	50
20	100	100
30	200	200

*For NIH3T3 and 293T cells 10 µl packed cell volume is equivalent to about 10^6 cells

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B. Adherent cell

1. Prior to protein extraction pre-chill buffers and the protein extraction filter cartridge in collection tube on ice.
2. Grow adherent cells to 90-100% confluence and wash the cells once in the tissue culture plates with cold PBS, aspirate the buffer completely.
3. Add appropriate amounts of buffer A (Table 2), swirl to distribute the buffer over the entire surface of tissue cultures; place the tissue culture on ice for 2 min and add an equal volume of buffer B. Mix well by scraping the lysed cells with a pipette tip or with a transfer pipette and transfer the cell lysate to a pre-chilled protein extraction filter cartridge in collection tube, centrifuge at top speed (14,000-16.000 rpm) in a microcentrifuge for 30 seconds to one min. The protein extract is now ready for downstream applications.

Table 2 Amounts of buffers required for different amount of adherent cells

Containers	Buffer A (µl)	Buffer B (µl)
96-well plate	25	25
24-well plate	125	125
6-well plate	250	250

Protein Extraction Procedures for Animal Tissues

Following procedures are for 10-20 mg starting animal tissues. For insect such as *Drosophila* use 15-20 *Drosophila* larvae, pupa or adult flies/sample. If smaller or larger amounts of starting materials are used adjust the amount of buffers proportionately.

1. Prior to protein extraction pre-chill buffers and the protein extraction filter cartridge in collection tube on ice.
2. Place 10-20 mg fresh/frozen tissue in the filter and add 200 µl buffer A to the filter. Grind the tissue with a plastic rod for 50-60 time with twisting force, add 200 µl buffer B 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 centrifuge at a microcentrifuge at top speed for 30 second to one min. The supernatant of flow through contains total protein extract. Transfer clear supernatant to a fresh tube (this is detergent-free total protein extract).

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