Minute™ Cytoplasmic and Nuclear Extraction Kit

Catalog number: SC-003

Description

Invent Biotechnologies Minute™Cytolpasmic and Nuclear Extraction Kit is composed of optimized cytoplamic extraction buffer, nuclear protein extraction buffer and protein extraction filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly separate native cytosol and nuclear proteins from cultured mammalian cells or tissues. Due to the use of the protein extraction filter cartridges separation of cytolasmic and nuclear proteins can be accomplished in less than 15 min.

Application

The kit is designed to rapidly extract native cytoplasmic and nuclear proteins from cultured cells or tissues for applications such as SDS-PAGE, immunoblottings, ELISA, IP, protein localization, gel mobility shift assays, 2-D gels and other applications. This kit provides the most rapid method currently available for fractionation of native cytoplasmic and nuclear proteins.

Buffer Formulation: Proprietary

Kit Components

1. 25 ml cytoplamic extraction buffer (Part#: SC-003-L)
2. 25 ml nuclear extraction buffer (Part#: SC-003-N)
3. 50 protein extraction cartridges (Part#: P-001)
4. 50 collection tubes with cap (Part#: P-002)

Storage

Store the kit at 4^{o}C upon arrival.

Important Product Information

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 inhibitor to extracted lysate is recommended. The nuclear extraction buffer contains 300 mM salt, for some applications, dilution or desalting of the extract may be needed. . For determination of protein concentration, BCA kit (Pierce) is recommended. The protein extraction filter cartridge has the capacity of 500 µl. Multiple filter cartridges may be used if larger amount of cell lysate is desired. To study protein phosphorylation, phosphatase inhibitors must 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)
Micro-Tube Pestles (RPI, Cat #: 199222XX, 299220)

A. Cultured Cells in suspension

1. Harvest cells in suspension by low speed centrifugation (500 X g for 3 min). Wash the cell in cold PBS once.
2. Transfer the cells to a 1.5 ml microcentrifuge tube and pellet the cells by centrifugation at 3000 rpm for 1 min; aspirate the supernatant completely.
3. Add appropriate amounts of cytoplasmic extraction buffer to cell pellets (Table 1), vortex the tube vigorously for 15 seconds, incubate on ice for 5 min and vortex briefly. Go to Cytoplasmic and Nuclear Protein Extraction Procedures below.

B. Adherent cells

1. Grow adherent cells to 90-100% confluence and wash the cells twice in the tissue culture plates, dishes or flasks with cold PBS, aspirate the buffer completely.
2. Add appropriate amounts of cytoplasmic extraction buffer (Table 2), 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 lysate to pre-chilled 1.5 ml microcentrifuge tube. Vortex the tube vigorously for 15 seconds. Go to Cytoplasmic and Nuclear Protein Extraction Procedures below.

C. Preparation of Tissues

1. Weight desired amount of tissue and place the tissue in a pre-chilled 1.5 ml microcentrifuge tube.
2. Wash the tissue once with cold PBS. Centrifuge the tissue at 3000 rpm for 1 min; remove supernatant and leave the pellet as dry as possible.
3. Homogenize the tissue with appropriate amounts of cytoplasmic extraction buffer (Table 3) using a micro-tube pestle or a micro-grinder. Remove non-homogenized tissue debris. Go to Cytoplasmic and Nuclear Protein Extraction Procedures below.

Table 1, Buffer volume for different packed cell volume

Packed Cell Volume (µl)	Cytoplasmic Extraction Buffer ((µl)	Nuclear Extraction Buffer ((µl)
5	50	25
10	100	50
20	200	100

50	500	250

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

Table 2, , Buffer Volume for Different Amount of Adherent Cells

Containers	Cytoplasmic Extraction Buffer ((µl)	Nuclear Extraction Buffer ((µl)
24-well plate	80	25
6-well plate	300	150
25 cm^2 flask	600	250

Table 3, Amounts of Buffers required for different amounts of Tissues

Amount of tissues (mg)	Cytoplasmic Extraction Buffer ((µl)	Nuclear Extraction Buffer ((µl)
5	50	25
10-15	100	50
15-20	200	100
20-30	500	250

Cytoplasmic and Nuclear Protein Extraction Procedures

1. Centrifuge the tube for 5 min at top speed in a microcentrifuge at 4°C.
2. Transfer the supernatant (cytosol fraction) to a fresh pre-chilled 1.5 ml tube. Add appropriate amounts of nuclear extraction buffer to the pellet, vortex vigorously for 15 seconds, incubate the tube on ice for one min. Repeat the 15 second vortexing and one min incubation 4 times.
3. Immediately transfer/pour the nuclear extract to a pre-chilled filter cartridge with collection tube and centrifuge at top speed (14,000-16,000 rpm) in a microcentrifuge for 30 seconds. Discard the filter cartridge according to your institution's waste disposal protocol. Store nuclear extract at -80°C until use. Typical protein yield is about 1.5-2.5 mg/ml.

Troubleshooting

Problem	Solution
Low protein concentration	Increase amounts of cells/tissues or decrease amount of cell lysis buffer
Low protein activity	Keep lysate cold/add protease inhibitors

Appendix: Additional product information

Fig. 1, Comparison of protein extraction efficiency. Twenty µl 293T cell pellets were extracted with Minute™Cytolpasmic and Nuclear Extraction Kit or name brand

Nuclear and Cytoplasmic Extract Kit. Total protein was obtained by repeated sonication.

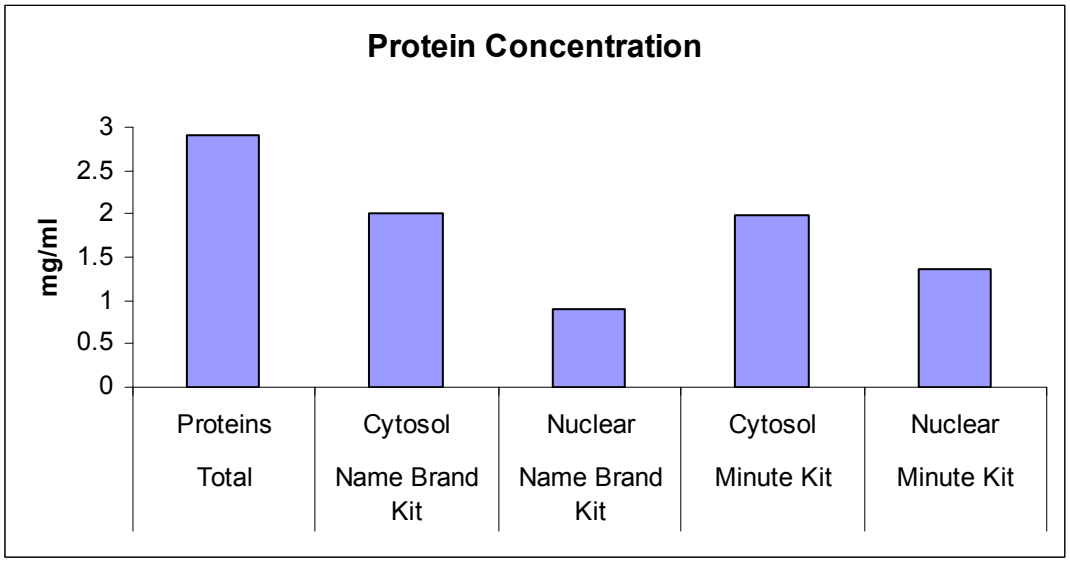
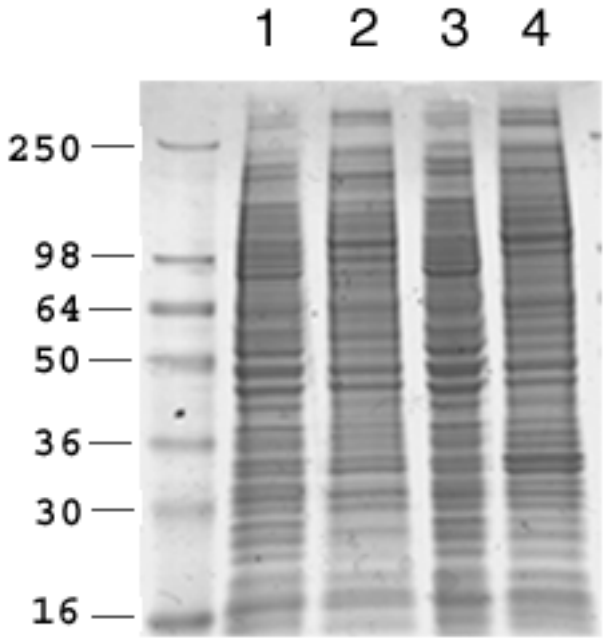


Fig. 2. Comparison of Minute™ Cytolpasmic and Nuclear Extraction Kit with name brand cytoplasmic and nuclear extraction kit.

A. Nuclear and cytoplasmic fractions were extracted from 20 µl 293T cell pellets; separated in 12% SDS-PAGE and stained with Coomassie blue. Lane 1, Cytoplasmic fraction (name brand kit). Lane 2, Nuclear fraction (name brand kit). Lane 3, Cytoplasmic fraction (Minute™ Kit), Lane 4, Nuclear fraction (Minute™ Kit).



B. Western Blotting. Proteins separated in 12% SDS-PAGE (shown in A) were transferred to nitrocellulose membrane and probed with anti- HDAC1 and actin.