Nuclear and Cytoplasmic Protein Extraction Kit

Catalog# BWR1008

Size: 50 tests

Lot # Check on the product label

Introduction

1. Nuclear and Cytoplasmic Protein Extraction Kit is designed to quickly prepare highly enriched fractions of cytoplasmic and nuclear proteins from eukaryotic samples, such as cultured cells and fresh tissues.
2. This kit provides a simple and convenient method for extracting nuclear and cytoplasmic protein. The user can complete the separation of nuclear and cytoplasmic protein within about 90 min. Extracted proteins can be directly apply for downstream reactions, such as Western, EMSA, foot printing, reporter gene detection, and measurement of enzyme activity.
3. Through Cytoplasmic Protein Extraction Reagent A and B, this kit breaks cell membrane and releases proteins for cell burst under hypotonic condition, then centrifuge for collection of the nucleoli. At last, extract the nuclear proteins by hyperhaline Nuclear Protein Extraction Reagent.
4. The kit can be used for 50 samples (60 mg / sample, or 2 $\times 10^6$ Hela cells). User can adjust the volume of reagent according to different sample volumes.

Kit Components

Components	Size	Storage Instruction
Cytoplasmic Protein Extraction Reagent A (CER A)	10 ml	Store at -20°C for one year.
Cytoplasmic Protein Extraction Reagent B (CER B)	1 ml	Store at -20°C for one year.
Nuclear Protein Extraction Reagent (NER)	3 ml	Store at -20°C for one year.

Protocol

1. Prepare solutions: thaw three reagents of the kit, put them on ice once completely dissolved, and mix thoroughly. Take the proper volume of CER A for use, add PMSF (2-3 min before use) to make its final concentration to 1 mM. Take the proper volume of NER for use, add PMSF (2-3 min before use) to make its final concentration to 1 mM.

2. For **adherent cells**: wash with PBS and scrape cells, or treat cells by EDTA, and stroke cells with pipette. Then centrifuge for several minutes to collect cells, discard supernatant, and keep cell pellets for use. Do not digest with pancreatin to avoid degradation of proteins.

3. For **suspending cells**: wash with PBS, then, centrifuge for several minutes to collect cells, discard supernatant, and keep cell pellets for use.

4. Add 200 μl of CER A (containing PMSF) into per 20 μl of cell pellets (the volume of 2×

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com

10^6 Hela cells is about 20 μl or 40 mg).

5. Vortex at maximum speed for 5 seconds to suspend cell pellets (If not complete, prolong the vortex time).

6. Incubate on ice for 10-15 min.

7. Add 10 μl of CER B. Vortex at maximum speed for 5 seconds, and incubate on ice for 1min.

8. Vortex at maximum speed for 5 seconds; then centrifuge at 12,000-16000 g at 4℃ for 5 min.

9. Immediately pipette the supernatant (cytoplasmic proteins) into a pre-cooling tube. Assay the cytoplasmic proteins immediately, or store at -70℃for use. (**Do not touch the precipitate, leave very small volume of supernatant to avoid touching**.)

10. For precipitate: discard the supernatant, add 50 μl of NER (containing PMSF) to the precipitate. (Discard the supernatant thoroughly to avoid contamination of cytoplasmic proteins.)

11. Vortex at maximum speed for 15-30 seconds to suspend pallets, put back on the ice and vortex for 15-30 seconds each1-2 min in following 30 minutes.

12. Centrifuge at 12,000-16,000 g at 4℃ for 10 min.

13. Immediately take the supernatant (nuclear proteins) into a new pre-cooling tube. Assay the nuclear proteins immediately, or store at -80℃.

14. For **fresh tissues**:

A. Cut the tissue into small slices. Mix the CER A and CER B at 20:1 (i.e. Add 10 μl of CER B into 200 μl of CER A). And add PMSF buffer to make its final concentration to1mM to prepare tissue homogenate solution. Add 200 μl of tissue homogenate solution to per 60 mg tissues, and homogenize completely with a glass homogenizer. Homogenization should be operated on ice or at 4℃.

B. Transfer the homogenate to a plastic tube, incubate on ice for 15 min.

C. Centrifuge at 1,500 g at 4℃ for 5 min, transfer supernatant (cytoplasmic proteins) to a pre-cooling tube (**Do not touch the precipitate, leave very small volume of supernatant to avoid touching**).

D. For precipitate: proceed Step 4. And then, proceed Step 5-13 to extract cytoplasmic and nuclear proteins. And the extracted cytoplasmic proteins can be combined with the proteins got by Step 14C.

Notes

1. PMSF (phenylmethylsulfonyl fluoride) buffer is not included in this product, we provide it separately (Catalog # BWR1014). Few minutes (2-3 min) advanced before use, add PMSF buffer to make its final concentration to1mM.
2. All steps of protein extraction should be operated on ice or at 4℃.
3. The Kit is designed for cultured cells and fresh tissue samples; it will not work efficiently for frozen tissue samples. The extracted protein of this kit can be assayed by our BCA Protein Assay Kits (Catalog # BWR1023, BWR1024, BWR1025, BWR1026).
4. Please wear the lab coat and disposable gloves to operate.

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com