Cytoplasmic and Nuclear Protein Extraction Kit

Catalog Number: WB-RR-90

Kit Components

Description	Quantity	Volume	Catalog Number
Cytoplasmic Extraction Reagent A (CER A)	1	30mL	WB-RR-90-A
Cytoplasmic Extraction Reagent B (CER B)	1	1.5mL	WB-RR-90-B
Nuclear Extraction Reagent (NER)	1	15mL	WB-RR-90-C

Overview

Product Name	Cytoplasmic and Nuclear Protein Extraction Kit
SKU/Catalog Number	WB-RR-90
Form	Liquid
Pack Size	1 kit
Assays per kit	60 assays for cell pellet fractions having packed cell volumes of 50µL each 30 assays for 0.1 g tissue
Storage	Upon receipt store Cytoplasmic and Nuclear Protein Extraction Kit at 4˚C. It is stable at 4˚C for one year. Product is shipped at ambient temperature.
Compatibility with reagents	Fully compatible with Broad Spectrum Protease Inhibitor Cocktail and Broad Spectrum Phosphatase Inhibitor Cocktail
Equivalent	Thermofisher (Product No. 78833, 78835), Millipore Sigma (Product No. NXTRACT)
Description	Cytoplasmic and Nuclear Protein Extraction Kit provides a complete set of extractionreagents that enable the separation of nuclear protein and cytoplasmic fractions from cultured cells and fresh tissues.

Assay Principle

This kit provides a complete set of extraction reagents that enable the separation of nuclear protein and cytoplasmic fractions from cultured cells and fresh tissues. The kit breaks cell membrane and release cytoplasmic proteins for cell burst under hypotonic condition. And then centrifuge for collection of the nucleoli. At last, extract nuclear proteins using Nuclear Extraction Reagent. Once desalted or diluted, the isolated soluble cytoplasmic proteins can be used to perform immunoassays and protein interaction experiments, such as EMSA, Co-IP and pull-down assays. Nuclear extracts are generally preferred to whole cell lysates for gene regulation studies. Cellular components present in whole cell lysates can adversely affect nuclear protein interactions and stability, and nuclear proteins are more concentrated in nuclear extracts than whole cell lysates.

Features of the Cytoplasmic and Nuclear Protein Extraction Kit:

Compatible—Extracted proteins can be directly apply for downstream assays, including Western blotting, gel-shift assays, protein assays, reporter gene assays and enzyme activity assays.

Fast—The optimized reagents and protocol allow non-denatured, active proteins to be purified in 90 minutes.

Convenient—simple instructions do not require ultracentrifugation over gradients.

Minimum cross-contamination—Cross-contamination of cytosolic proteins into the membrane fractions is usually about 10%.

Important Product Information

1. All steps of protein extraction should be operated on ice or at 4 ˚C.
2. The kit is designed for fresh tissue samples only. It will not work efficiently for frozen tissue samples.
3. Use BCA protein Assay kit to quantify isolated proteins.
4. If more concentrated nuclear extracts are desired, the volume of NER used in the extractions can be decreased 2- to 4-fold without adverse effects on protein recovery or compartmentalization.
5. If large volumes of nuclear extract are required in subsequent applications or if problems occur with downstream assays, dialyze the nuclear extract to remove excess salts before use.
6. Include protease inhibitors to maintain extract integrity and function.

Additional Materials Required

- Protease inhibitor and phosphatase inhibitor
- 2mL microcentrifuge tubes
- Vortex mixer
- Microcentrifuge capable of spinning at 16,000 x g
- Tissue homogenizer
- Phosphate-buffered saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2

Procedure for Cytoplasmic and Nuclear Protein Extraction from Different Sample Types

Reagent Preparation:

Place Cytoplasmic Extraction Reagent A (CER A), Cytoplasmic Extraction Reagent B (CER B) and Nuclear Extraction Reagent (NER) on ice. For optimal results, include protease inhibitor and phosphatase inhibitor before use.

Protocol 1: Adherent Cells & Suspension Cells

Cell Culture Preparation

1. **For adherent cells:** scrape the cells off the surface of the plate with a cell scraper. Centrifuge harvested cells at 600 x g for 5 minutes. Carefully remove and discard the supernatant, and keep cell pellets for use.
2. **For suspension cells:** centrifuge harvested cells at 600 x g for 5 minutes. Carefully remove and discard the supernatant, and keep cell pellets for use.
3. Resuspend the cells in pre-cooling PBS.
4. Transfer the cells to a 2mL microcentrifuge tube. Centrifuge at 600 x g for 5 minutes. Carefully remove and discard the supernatant, and keep cell pellets for use.
5. Add CER A to the cell pellet according to the volumes indicated in Table 1.

Table 1. Reagent volumes for different packed cell volumes

Packed cell Volume (μL)	CER A (μL)	CER B (μL)	NER (μL)
10	100	5	50
20	200	10	100
50	500	25.5	250
100	1000	50	500

The volume of 2×10^6 Hela cells is about 20μL.

6. Vortex the tube at maximum speed for 15 seconds to obtain a homogeneous cell suspension. Incubate on ice for 10-15 minutes.
7. Add CER B to the tube. Vortex the tube at maximum speed for 5 seconds. Incubate on ice for 1 minute. (If there is cytoplasmic protein in extracted nuclear protein, prolong the vortex time by 1-5 minutes.)
8. Vortex the tube at maximum speed for 5 seconds. Centrifuge the tube at 16000 × g for 5 minutes.
9. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.
10. Add NER to the insoluble cell debris containing nuclei produced in step 8.
11. Vortex at maximum speed for 5 seconds to obtain a homogeneous cell suspension (If not complete, prolong the vortex time). Place the cell debris on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 40 minutes.
12. Centrifuge the tube at 16000 × g for 5 minutes.
13. Immediately transfer the supernatant containing nuclear proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.

Protocol 2: Tissue

Tissue Preparation:

1. Place the fresh tissue into pre-chilled PBS and rinse several times. Dry the tissue with filter paper. Mince the tissue into small pieces and weigh the tissue sample.
2. Place tissue in a tissue homogenizer. Add CER A to the tissue according to the volumes indicated in Table 2.

Table 2. Reagent volumes for different tissue amounts

Tissue Weight (mg)	CER A (µL)	CER B (µL)	NER (µL)
20	200	10	100
40	400	20	200
80	800	40	400
100	1000	50	500

3. Homogenize tissue on ice to obtain a homogeneous suspension. Transfer the homogeneous suspension to a 2mL microcentrifuge tube. Incubate on ice for 10 minutes.
4. Add CER B to the tube. Vortex the tube at maximum speed for 5 seconds. Incubate on ice for 1 minute.
5. Vortex the tube at maximum speed for 5 seconds. Centrifuge the tube at 16000 × g for 5 minutes.
6. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.
7. Add NER to the insoluble cell debris containing nuclei produced in step 5.
8. Vortex at maximum speed for 5 seconds to obtain a homogeneous cell suspension (If not complete, prolong the vortex time). Place the cell debris on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 40 minutes.
9. Centrifuge the tube at 16000 × g for 5 minutes.
10. Immediately transfer the supernatant containing nuclear proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.

Note:
For some tissues, if the cytoplasmic or nuclear proteins fail to be extracted as expected, follow Protocol 3.

Protocol 3: Tissue

1. Place the fresh tissue into pre-chilled PBS and rinse several times. Dry the tissue with filter paper. Mince the tissue into small pieces and weigh the tissue sample.
2. Place tissue in a tissue homogenizer.
3. Mix CER A with CER B at a volume ratio of 20:1 to generate CER A and CER B Mixture. e.g. Add 10µL of CER B into 200µL of CER A.
4. Add CER A and CER B Mixture to the tissue at a ratio of 10:1(v/w). e.g. Add 200µL of CER A and CER B Mixture to 20mg tissue.
5. Homogenize tissue on ice to obtain a homogeneous suspension. Transfer the tissue homogenate to a 2mL microcentrifuge tube. Incubate on ice for 15 minutes.
6. Centrifuge the tube at 1500 × g for 5 minutes at 4 ˚C.

7. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use. (These are partial cytoplasmic proteins extracted from the tissue sample. Do not touch the pellet when aspirating the supernatant. There are still many cells in the pellet that have not been broken.)
8. Add CER A to the cell pellet according to the volumes indicated in Table 3.

Table 3. Reagent volumes for different packed cell volumes

Packed cell Volume (μL)	CER A (μL)	CER B (μL)	NER (μL)
10	100	5	50
20	200	10	100
50	500	25.5	250
100	1000	50	500

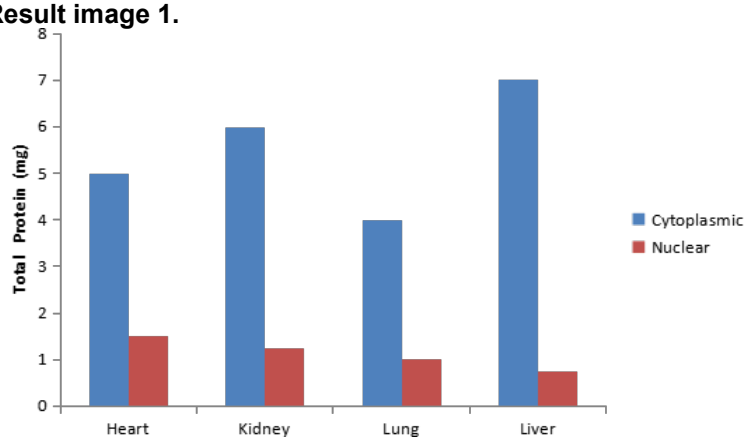
The volume of 2×10^6 Hela cells is about 20μL.

9. Vortex the tube at maximum speed for 15 seconds to obtain a homogeneous cell suspension. Incubate on ice for 10-15 minutes.
10. Add CER B to the tube. Vortex the tube at maximum speed for 5 seconds. Incubate on ice for 1 minute.
11. Vortex the tube at maximum speed for 5 seconds. Centrifuge the tube at 16000 × g for 5 minutes.
12. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.
13. Add NER to the insoluble cell debris containing nuclei produced in step 8.
14. Vortex at maximum speed for 5 seconds to obtain a homogeneous cell suspension (If not complete, prolong the vortex time). Place the cell debris on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 40 minutes.
15. Centrifuge the tube at 16000 × g for 5 minutes.
16. Immediately transfer the supernatant containing nuclear proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.

Note: Cytoplasmic proteins produced in Step 6 and Step 11 can be stored and used together.

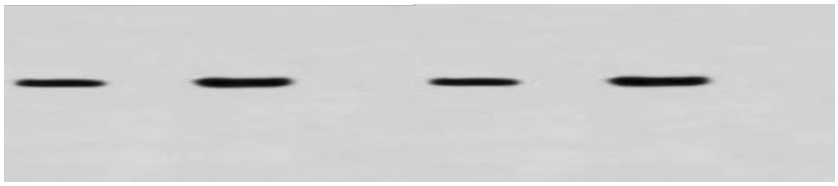
Result Images

Result image 1.



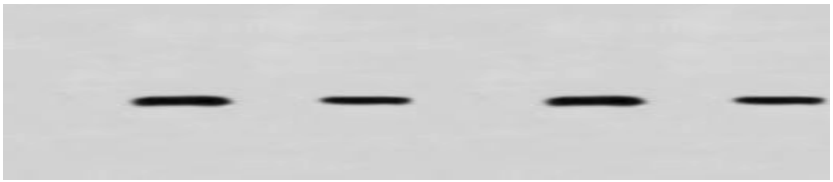
Nuclear proteins and cytoplasmic proteins were extracted from mouse heart, kidney, lung and liver tissues (100mg) with Cytoplasmic and Nuclear Protein Extraction Kit. BCA protein Assay kit was used to quantify isolated nuclear and cytoplasmic proteins.

Result image 2.
GAPHD



CE NE Heart | CE NE Kidney | CE NE Lung | CE NE Liver

PCNA



CE NE Heart | CE NE Kidney | CE NE Lung | CE NE Liver

Nuclear proteins (NE) and cytoplasmic proteins (CE) were extracted from mouse heart, kidney, lung and liver tissues with Cytoplasmic and Nuclear Protein Extraction Kit. Proteins were used to perform WB for GAPDH and PCNA expressed in cytoplasm and in nuclei.

Problem	Possible Cause	Solution
Low cytoplasmic protein yield	Cells were not lysed completely	Increase amount of CER A and CER B Reagent
	Cell pellet was not dispersed	Vortex thoroughly
	Tissues was not homogenized sufficiently	Homogenize sufficiently
Low nuclear protein yield	Cell pellet was not dispersed	Vortex thoroughly
	Incomplete nuclei isolation	Increase time of centrifugation
No or low protein activity detected	Samples were not kept cold	Keep samples on ice between vortexing steps
	Presence of protease	Use a protease inhibitor cocktail
Proteins not compartmentalized	Extraction time for cytoplasmic protein is too long.	Decrease extraction time for cytoplasmic protein.
	Incomplete removal of cytoplasmic extract	Carefully remove all cytoplasmic extract before nuclear lysis
	Cytoplasmic extract contains some nuclear precipitation while transferring the supernatant containing cytoplasmic proteins	Carefully remove all cytoplasmic extract before nuclear lysis