TOOLS Cytoplasmic and Nuclear Protein Extraction Kit

For the separation of nuclear proteins and cytoplasmic fractions

Contents

Introduction

This kit provides a complete set of extraction reagents that enables the separation of nuclear proteins and cytoplasmic fractions from cultured cells and fresh tissues. The kit functions by breaking the cell membrane and releasing cytoplasmic proteins for cell bursting under hypotonic conditions before the subsequent collection of the nucleoli through centrifuging. Finally, the nuclear proteins are extracted using the provided Nuclear Extraction Reagent. Once desalted or diluted, the isolated soluble proteins can be used for immunoassays and protein interaction experiments, such as EMSA, Co-IP, and pull-down assays.

Features

1. Superior compatibility: extracted proteins can be directly applied in downstream assays, including Western blotting, gel-shift assays, protein assays, reporter gene assays, and enzyme activity assays.
2. Fast: the optimized reagents and protocol allow nondenatured, active proteins to be purified within 90 min.
3. Convenient: ultracentrifugation over gradients is not required.
4. Minimum cross-contamination: The cross-contamination of cytosolic proteins into the membrane fractions is typically approximately 10%.

Important Product Information

1. All protein extraction steps should be performed on ice or at 4°C
2. The kit is designed for fresh tissue samples only. It will not operate efficiently for frozen tissue samples.
3. Use the TOOLS BCA protein assay kit (Product No.: TAAR-ZBE6 or TAAR-TB0) to quantify isolated proteins.
4. If more concentrated nuclear extracts are desired, the volume of the Nuclear Extraction Reagent (NER) used in the extractions can be decreased 2- to 4-fold with no 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.

Kit Contents

Contents	BRARZ106
Cytoplasmic Extraction Reagent A (CER A)	30 mL
Cytoplasmic Extraction Reagent B (CER B)	1.5 mL
Nuclear Extraction Reagent (NER)	15 mL

The kit contains sufficient extraction reagents either for extracting 60 cell pellet fractions with each having a packed cell volume of 50 µL or for extracting 30 tissue fractions, each weighing 0.1 g.

Storage

4°C for 1 year

Additional Materials Required

1. Protease inhibitor (Product No.: TAAR-BBI2) and phosphatase inhibitor (Product No.: TAAR-BBI3)
2. 2-mL microcentrifuge tubes
3. Vortex mixer
4. Microcentrifuge capable of spinning at 16000 × g
5. Tissue homogenizer
6. PBS: 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2

Protocol

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 the protease inhibitor and phosphatase inhibitor before use.

A. 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 × g for 5 min. Carefully remove and discard the supernatant and keep cell pellets for use.
2. For suspension cells: centrifuge harvested cells at 600 × g for 5 min.
   Carefully remove and discard the supernatant and keep cell pellets for use.
3. Resuspend the cells in precooling PBS.
4. Transfer the cells to a 2-mL microcentrifuge tube. Centrifuge at 600 × g for 5 min. 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 5 s (or for as long as required) to obtain a homogeneous cell suspension. Incubate on ice for 10 min.
7. Add CER B to the tube. Vortex the tube at maximum speed for 5 s. Incubate on ice for 1 min.
8. Vortex the tube at maximum speed for 5 s. Centrifuge the tube at 16000 × g for 5 min.
9. Immediately transfer the supernatant-containing cytoplasmic proteins to a clean prechilled tube. Place this tube on ice until use or store the aliquots at −80°C for future use.
10. Add NER to the nuclei-containing insoluble cell debris produced in step 8.
11. Vortex at maximum speed for 5 s (or for as long as required) to obtain a homogeneous cell suspension. Place the cell debris on ice and continue vortexing for 15 s. Vortex the mixture five times (15 s each time), with a 10-min interval between each vortex.
12. Centrifuge the tube at 16000 × g for 5 min.
13. Immediately transfer the supernatant-containing nuclear proteins to a clean prechilled tube. Place this tube on ice until use or store the aliquots at −80°C for future use.

B. Tissue

Tissue Preparation

1. Place the fresh tissue into prechilled PBS and rinse several times. Dry the tissue with filter paper. Mince the tissue into small pieces and weigh the tissue sample.
2. Place the 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. Vortex the tube at maximum speed for 5 s (or for as long as required) to obtain a homogeneous cell suspension. Incubate on ice for 10 min.
4. Add CER B to the tube. Vortex the tube at maximum speed for 5 s. Incubate on ice for 1 min.
5. Vortex the tube at maximum speed for 5 s. Centrifuge the tube at 16000 × g for 5 min.
6. Immediately transfer the supernatant-containing cytoplasmic proteins to a clean prechilled tube. Place this tube on ice until use or store the aliquots at −80°C for future use.
7. Add NER to the nuclei-containing insoluble cell debris produced in step 8.
8. Vortex at maximum speed for 5 s (or for as long as needed) to obtain a homogeneous cell suspension. Place the cell debris on ice and continue vortexing for 15 s every 10 min. Vortex the mixture five times (15 s each time), with a 10-min interval between each vortex.
9. Centrifuge the tube at 16000 × g for 5 min.
10. Immediately transfer the supernatant-containing nuclear proteins to a clean prechilled tube. Place this tube on ice until use or store the aliquots at −80°C for future use.

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