INSTRUCTIONS

Subcellular Protein Fractionation Kit for Cultured Cells

78840

2131.4

Number	Description
78840	**Subcellular Protein Fractionation Kit for Cultured Cells**, sufficient reagents for extracting 50 cell pellet fractions having packed cell volumes of 20µL each (a total of ~2g cell paste)
	Kit Contents:
	Cytoplasmic Extraction Buffer (CEB), 10mL, store at -20°C
	Membrane Extraction Buffer (MEB), 10mL, store at 4°C
	Nuclear Extraction Buffer (NEB), 10mL, store at 4°C
	Pellet Extraction Buffer (PEB), 5mL, store at room temperature
	Micrococcal Nuclease, ≥ 100 units/µL, 150µL, store at -20°C
	Calcium Chloride ($CaCl_2$), 100mM, 250µL, store at 4°C
	Halt™ Protease Inhibitor Cocktail, 100X, 350µL, store at 4°C
	Storage: Upon receipt store kit at -20°C, or store individual components as indicated above. Kit is shipped with dry ice.

Introduction

The Thermo Scientific Subcellular Protein Fractionation Kit for Cultured Cells enables stepwise separation and preparation of cytoplasmic, membrane, nuclear soluble, chromatin-bound and cytoskeletal protein extracts from mammalian cultured cells. The first reagent added to a cell pellet causes selective cell membrane permeablization, releasing soluble cytoplasmic contents. The second reagent dissolves plasma, mitochondria and ER/golgi membranes but does not solubilize nuclear membranes. After recovering the intact nuclei by centrifugation, a third reagent yields the soluble nuclear extract. A second nuclear extraction with micrococcal nuclease is performed to release chromatin-bound nuclear proteins. The recovered insoluble pellet is then extracted with the final reagent to isolate cytoskeletal proteins.

Extracts obtained with the Subcellular Protein Fractionation Kit for Cultured Cells are compatible with a variety of downstream applications including Western blotting, Thermo Scientific Pierce BCA Protein Assay (Product No. 23225), Thermo Scientific LightShift Chemiluminescent EMSA Kit (Product No. 20148), and reporter-gene and enzyme-activity assays. Extracts from each subcellular compartment generally have less than 15% contamination between fractions, which is sufficient purity for most experiments studying protein localization and redistribution.

Important Procedural Notes

Note: For tissue samples, use the Thermo Scientific Subcellular Protein Fractionation Kit for Tissue (Product No. 87790).

- Thaw all buffers using a room temperature water bath, and keep CEB, MEB and NEB on ice until use. If precipitate occurs in PEB, mix vigorously to resuspend. Presence of a precipitate does not adversely affect PEB performance.
- Protease inhibitors are required to maintain extract integrity and function. Immediately before use, add protease inhibitors to CEB, MEB, NEB and PEB by diluting Thermo Scientific Halt Protease Inhibitor Cocktail 1:100 into each volume of buffer required.
- Perform all incubations at 4°C unless otherwise noted. Use a rotary shaker to avoid clumping of insoluble material during incubations.
- Perform all centrifugation steps at 4°C. Keep cell samples and extracts on ice unless otherwise noted.
- Subcellular protein extracts can be used directly in many downstream assays. Some applications might require dialysis or desalting to remove detergent and salts. Although the detergent in the MEB is not dialyzable, it does not interfere with isoelectric focusing. PEB contains a strong denaturing detergent that is not compatible with isoelectric focusing. For 2D analysis of cytoskeletal proteins, resuspend pellet directly in 2D sample buffer.

Additional Materials Required

- Ice-cold phosphate-buffered saline (PBS): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372)
- Rotary shaker in cold room
- Microcentrifuge at 4°C

Cell Culture Preparation

1. For adherent cells, harvest with trypsin-EDTA and then centrifuge at 500 × *g* for 5 minutes. For suspension cells, harvest by centrifuging at 500 × *g* for 5 minutes.
2. Wash cells by suspending the cell pellet with ice-cold PBS.
3. Transfer 1-10 × 10^6 cells to a 1.5mL microcentrifuge tube and pellet by centrifugation at 500 × *g* for 2-3 minutes.
4. Use a pipette to carefully remove and discard the supernatant, leaving the cell pellet as dry as possible.
5. Add ice-cold CEB containing protease inhibitors to the cell pellet (Table 1). Proceed to Subcellular Protein Extraction, using the reagent volumes indicated in Table 1.

Table 1. Reagent volumes for different packed cell volumes.*

Packed Cell Volume (µL)	CEB (µL)	MEB (µL)	NEB (µL)	NEB (µL) +$CaCl_2$, MNase†	PEB (µL)
10	100	100	50	50	50
20	200	200	100	100	100
50	500	500	250	250	250
100	1000	1000	500	500	500

*For HeLa cells, 2 × 10^6 cells is equivalent to 20µL packed cell volume. †MNase = Micrococcal Nuclease

Subcellular Protein Fractionation

Note: Scale this protocol depending on the cell pellet volume (Table 1). Maintain the volume ratio of CEB:MEB:NEB:PEB reagents at 200:200:100:100µL, respectively.

1. After adding CEB to the cell pellet, incubate the tube at 4°C for 10 minutes with gentle mixing.
2. Centrifuge at 500 × *g* for 5 minutes. Immediately transfer the supernatant (cytoplasmic extract) to a clean pre-chilled tube on ice.
3. Add ice-cold MEB containing protease inhibitors to the pellet. Vortex the tube for 5 seconds on the highest setting. Incubate tube at 4°C for 10 minutes with gentle mixing.
4. Centrifuge at 3000 × *g* for 5 minutes.
5. Transfer the supernatant (membrane extract) to a clean pre-chilled tube on ice.
6. Add ice-cold NEB containing protease inhibitors to the pellet. Vortex on the highest setting for 15 seconds. Incubate tube at 4°C for 30 minutes with gentle mixing.
7. Centrifuge at 5000 × *g* for 5 minutes. Transfer the supernatant (soluble nuclear extract) fraction to a clean pre-chilled tube on ice.
8. Prepare chromatin-bound extraction buffer by adding 5µL of 100mM $CaCl_2$ and 3µL of Micrococcal Nuclease (300 units) per 100µL of room temperature NEB.
9. Add room temperature NEB containing protease inhibitors, $CaCl_2$ and Micrococcal Nuclease to the pellet. Vortex on the highest setting for 15 seconds.
10. Incubate at room temperature for 15 minutes or in a 37°C water bath for 5 minutes.
11. After incubation, vortex on the highest setting for 15 seconds and centrifuge the tube at 16,000 × *g* (highest setting of microcentrifuge) for 5 minutes.
12. Transfer the supernatant (chromatin-bound nuclear extract) fraction to a clean pre-chilled tube on ice.

13. Add room temperature PEB containing protease inhibitors to the pellet. Vortex on the highest setting for 15 seconds. Incubate at room temperature for 10 minutes.
14. Centrifuge the tube at 16,000 × *g* (i.e., the highest microcentrifuge setting) for 5 minutes. Transfer the supernatant (i.e., the cytoskeletal extract) to a new tube.

    Note: For same-day use, maintain fractions on ice for downstream applications and analysis. For long-term storage, store fractions at -80°C.

Troubleshooting

Problem	Possible Cause	Solution
Low cytoplasmic protein yield	Cells not lysed	Increase incubation time in CEB
	Cell pellet not dispersed	Mix cells gently during incubation
	CEB stored improperly	Store CEB at -20°C
Low membrane protein yield	Membranes solubilized with CEB	Decrease incubation time in CEB
	Incomplete membrane protein isolation	Increase time in MEB
Low soluble nuclear protein yield	Nuclei not extracted	Vortex thoroughly
	Incomplete nuclei isolation	Increase time of centrifugation after adding MEB
Low chromatin-bound protein yield	Calcium chloride or micrococcal nuclease not added	Add $CaCl_2$ and micrococcal nuclease to NEB before extraction
	Micrococcal nuclease stored improperly	Store micrococcal nuclease at -20°C
	Chromatin not completely degraded	Vortex thoroughly, add more micrococcal nuclease or incubate longer at 37°C
Low overall protein yield	Volumes of extraction reagents were not appropriate for given packed cell volume	Use the reagent volumes listed in Table 1
Proteins not compartmentalized	Incomplete lysis	Remove all PBS before adding CEB
		Vortex longer to completely disperse the pellet
		Increase incubation time
	Incomplete removal of extracts	Carefully remove all extract before proceeding to the next step
		Re-centrifuge sample and remove excess extract
		Rinse pellets with additional extraction buffers or PBS

Related Thermo Scientific Products

87790 **Subcellular Protein Fractionation Kit for Tissue**

87791 **Pierce Tissue Strainers, 50/pkg**

87785 **Halt Protease Inhibitor Cocktail, EDTA Free (100X)**

87786 **Halt Protease Inhibitor Cocktail (100X)**

88660 **Pierce Protease Inhibitor Tablets, 30 pack**

88661 **Pierce Protease Inhibitor Tablets, EDTA-free, 30 pack**

88216 **Micrococcal Nuclease, ≥ 100 units/µL,** 150µL

78833 **NE-PER® Nuclear and Cytoplasmic Extraction Reagents,** 1 kit

23224 **Pierce® BCA Protein Assay Reagent Kit**

22660 **Pierce 660nm Protein Assay Reagent**

69558 **Slide-A-Lyzer® MINI Dialysis Units plus Float, 3.5K MWCO,** 10 units

89882 **Zeba™ Spin Desalting Columns, 0.5mL,** 25/pkg

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Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce