NE-PER® Nuclear and Cytoplasmic Extraction Reagents

78833 78835

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Number	Description
78833	**NE-PER Nuclear and Cytoplasmic Extraction Reagents**, 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 Reagent I (CER I),** 10mL **Cytoplasmic Extraction Reagent II (CER II),** 550µL **Nuclear Extraction Reagent (NER),** 5mL
78835	**NE-PER Nuclear and Cytoplasmic Extraction Reagents**, sufficient reagents for extracting 250 cell pellet fractions having packed cell volumes of 20µL each (a total of ~10g cell paste) **Kit Contents:** **Cytoplasmic Extraction Reagent I (CER I),** 50mL **Cytoplasmic Extraction Reagent II (CER II),** 2.75mL **Nuclear Extraction Reagent (NER),** 25mL
	Storage: Upon receipt store kit components at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Reagents enable stepwise separation and preparation of cytoplasmic and nuclear extracts from mammalian cultured cells or tissue. Non-denatured, active proteins are purified in less than two hours. Addition of the first two reagents to a cell pellet causes cell membrane disruption and release of cytoplasmic contents. After recovering the intact nuclei from the cytoplasmic extract by centrifugation, the nuclei are lysed with a third reagent to yield the nuclear extract. Extracts obtained with this product generally have less than 10% contamination between nuclear and cytoplasmic fractions, which is sufficient purity for most experiments involving nuclear extracts.

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. Nuclear extracts obtained with the NE-PER Reagents are compatible with a variety of downstream applications including Western blotting, the Thermo Scientific Pierce BCA Protein Assay (Product No. 23225), gel-shift (Product No. 20148), reporter-gene and enzyme-activity assays.

Important Product Information

- Use protease inhibitors to maintain extract integrity and function. Immediately before use, add protease inhibitors to CER I and NER from concentrated stocks (e.g., 100X) to minimize reagent dilution. It is unnecessary to add protease inhibitors to CER II.
- 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. The detergent in the NE-PER Reagents is not dialyzable, but it will be primarily in the cytoplasmic fractions. For dialysis, use a Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit. Alternatively, 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.
- Perform all centrifugation steps at 4°C. Keep cell samples and extracts on ice.

Additional Materials Required

- Protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Cocktail, Product No. 78425 or 78437)
- Phosphate-buffered saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372)

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 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 CER I to the cell pellet (Table 1). Proceed to Cytoplasmic and Nuclear Protein Extraction, using the reagent volumes indicated in Table 1.

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

Packed Cell Volume (µL)	CER I (µL)	CER II (µL)	NER (µL)
10	100	5.5	50
20	200	11	100
50	500	27.5	250
100	1000	55	500

*For HeLa cells, 2 × 10^6 cells is equivalent to 20µL packed cell volume.

Tissue Preparation

1. Cut 20-100mg of tissue into small pieces and place in a microcentrifuge tube.
2. Wash tissue with PBS. Centrifuge tissue at 500 × *g* for 5 minutes.
3. Using a pipette, carefully remove and discard the supernatant, leaving cell pellet as dry as possible.
4. Homogenize tissue using a Dounce homogenizer or a tissue grinder in the appropriate volume of CER I (Table 2). Proceed Cytoplasmic and Nuclear Protein Extraction, using the reagent volumes indicated in Table 2.

Table 2. Reagent volumes for different tissue amounts.*

Tissue Weight (mg)	CER I (µL)	CER II (µL)	NER (µL)
20	200	11	100
40	400	22	200
80	800	44	400
100	1000	55	500

*Different tissue types may require more or less NE-PER Reagents per weight to optimally extract cytoplasmic and nuclear proteins.

Cytoplasmic and Nuclear Protein Extraction

Note: Scale this protocol depending on the cell pellet volume (Tables 1 and 2). Maintain the volume ratio of CER I:CER II:NER reagents at 200:11:100µL, respectively.

1. Vortex the tube vigorously on the highest setting for 15 seconds to fully suspend the cell pellet. Incubate the tube on ice for 10 minutes.
2. Add ice-cold CER II to the tube.
3. Vortex the tube for 5 seconds on the highest setting. Incubate tube on ice for 1 minute.
4. Vortex the tube for 5 seconds on the highest setting. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge (~16,000 × *g*).
5. Immediately transfer the supernatant (cytoplasmic extract) to a clean pre-chilled tube. Place this tube on ice until use or storage (see Step 10).

6. Suspend the insoluble (pellet) fraction produced in Step 4, which contains nuclei, in ice-cold NER.
7. Vortex on the highest setting for 15 seconds. Place the sample on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 40 minutes.
8. Centrifuge the tube at maximum speed (~16,000 × *g*) in a microcentrifuge for 10 minutes.
9. Immediately transfer the supernatant (nuclear extract) fraction to a clean pre-chilled tube. Place on ice.
10. Store extracts at -80°C until use.

Troubleshooting

Problem	Possible Cause	Solution
Low cytoplasmic protein yield	Cells were not lysed	Increase amount of CER II Reagent
	Cell pellet was not dispersed	Vortex thoroughly
	Tissues were homogenized in PBS	Homogenize tissues in CER I
Low nuclear protein yield	Cell pellet was not dispersed	Vortex thoroughly
	Incomplete nuclei isolation	Increase time of centrifugation following addition of CER II
Low protein concentration	Volumes of extraction reagents were not appropriate for given packed cell volume or tissue weight	Use the reagent volumes as directed in Tables 1 or 2
No or low protein activity detected	Samples were not kept cold	Centrifuge at 4°C and keep samples on ice between vortexing steps
	Presence of proteases	Use a protease inhibitor cocktail
Proteins not compartmentalized	Incomplete lysis of cells	Remove all PBS before adding CER I
		Increase vortexing time to adequately disperse the cell pellet
		Increase recommended incubation times
	Incomplete removal of cytoplasmic extract	Carefully remove all cytoplasmic extract before nuclear lysis
		Centrifuge sample and remove excess cytoplasmic extract
		Rinse nuclei with additional CER I buffer or PBS
	Over-, under- or non-uniform homogenization of tissue	Optimize tissue homogenization time and conditions

Related Thermo Scientific Products

78425 **Halt™ Protease Inhibitor Cocktail Kit**

78437 **Halt Protease Inhibitor Cocktail Kit, EDTA-Free**

20148 **LightShift Chemiluminescent EMSA Kit**

23225 **Pierce BCA Protein Assay Reagent Kit**

23235 **Micro BCA™ Protein Assay Reagent Kit**

69570 **Slide-A-Lyzer® MINI Dialysis Units**

28372 **BupH™ Phosphate Buffered Saline Pack,** 40 packs

78840 **Subcellular Protein Fractionation Kit**

22660 **Pierce 660nm Protein Assay Reagent**

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

General References

Nien-Pei, Tsai, *et al*. (2010). Dual action of epidermal growth factor: extracellular signal-stimulated nuclear-cytoplasmic export and coordinated translation of selected messenger RNA. *J Cell Biol* **188(3)**, 325-3.

Smirnova, I.V., *et al.* (2000). Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. *J. Biol Chem.* **275(13)**, 9377-9384.