NE-PER Nuclear and Cytoplasmic Extraction Reagents

78833  78835

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>78833</td>
<td>NE-PER Nuclear and Cytoplasmic Extraction Reagents, sufficient reagents for extracting 50 cell pellet fractions having packed cell volumes of 20 µL each (a total of ~2 g cell paste)</td>
</tr>
<tr>
<td></td>
<td>Kit Contents:</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic Extraction Reagent I (CER I), 10 mL</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic Extraction Reagent II (CER II), 550 µL</td>
</tr>
<tr>
<td></td>
<td>Nuclear Extraction Reagent (NER), 5 mL</td>
</tr>
<tr>
<td>78835</td>
<td>NE-PER Nuclear and Cytoplasmic Extraction Reagents, sufficient reagents for extracting 250 cell pellet fractions having packed cell volumes of 20 µL each (a total of ~10 g cell paste)</td>
</tr>
<tr>
<td></td>
<td>Kit Contents:</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic Extraction Reagent I (CER I), 50 mL</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic Extraction Reagent II (CER II), 2.75 mL</td>
</tr>
<tr>
<td></td>
<td>Nuclear Extraction Reagent (NER), 25 mL</td>
</tr>
</tbody>
</table>

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 proteins are extracted out of the nuclei with the third reagent. 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

- This kit is intended for use with fresh (not frozen) cells or tissue samples. 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 Device. 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 × 10⁶ 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>
<thead>
<tr>
<th>Packed Cell Volume (µL)</th>
<th>CER I (µL)</th>
<th>CER II (µL)</th>
<th>NER (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100</td>
<td>5.5</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>500</td>
<td>27.5</td>
<td>250</td>
</tr>
<tr>
<td>100</td>
<td>1000</td>
<td>55</td>
<td>500</td>
</tr>
</tbody>
</table>

*For HeLa cells, 2 × 10⁶ 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>
<thead>
<tr>
<th>Tissue Weight (mg)</th>
<th>CER I (µL)</th>
<th>CER II (µL)</th>
<th>NER (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>200</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>400</td>
<td>22</td>
<td>200</td>
</tr>
<tr>
<td>80</td>
<td>800</td>
<td>44</td>
<td>400</td>
</tr>
<tr>
<td>100</td>
<td>1000</td>
<td>55</td>
<td>500</td>
</tr>
</tbody>
</table>

*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. Centrifugate 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

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

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 Devices
- 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


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