INSTRUCTIONS



N-PERTM Neuronal Protein Extraction Reagent

87792

Number Description

87792 N-PER Neuronal Protein Extraction Reagent, 100mL, sufficient reagent to extract protein from

~10g of cells or tissue

Storage: Upon receipt store product at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific N-PER Neuronal Protein Extraction Reagent is a unique reagent optimized for removing neuronal proteins from neuronal tissue and primary neuronal cells. The prepared lysate can be used for enzymatic activity assays (e.g., phosphatase, kinase, ATPase), immunoassays (e.g., Western blot, ELISA, RIA) and protein purifications. Additionally, the reagent is compatible with standard protein estimation assays such as the Thermo Scientific Pierce BCA Protein Assay (Product No. 23225).

Procedure for Neuronal Tissue Protein Extraction

Note: N-PER Reagent does not contain protease or phosphatase inhibitors. If required, add protease inhibitors, phosphatase inhibitors, reducing agents or chelating agents to the N-PER Reagent just before use (see the Related Thermo Scientific Products Section).

- 1. Harvest neuronal tissue and wash with phosphate-buffered saline (PBS, Product No. 28372). Keep tissue on ice if used immediately; otherwise, freeze tissue in liquid nitrogen and store at -80°C.
- 2. Tissue samples must be \geq 50mg. Weigh the sample and use a ratio of 1g of tissue to 10mL of N-PER Reagent.
- 3. Add the appropriate amount of N-PER Reagent to the tissue sample and homogenize. For best protein yield, use Dounce homogenization and 10-20 strokes on ice. Transfer homogenate to an appropriate microcentrifuge tube(s). Incubate on ice for 10 minutes.
- 4. Centrifuge the sample at $10,000 \times g$ for 10 minutes at 4°C to pellet the cell debris.
- 5. Collect supernatant and continue with downstream analysis or further purification.

Procedure for Neuronal Primary Cell Protein Extraction

Note: N-PER Reagent does not contain protease or phosphatase inhibitors. If required, add protease inhibitors, phosphatase inhibitors, reducing agents or chelating agents to the N-PER Reagent just before use (see the Related Thermo Scientific Products Section).

- 1. Carefully remove (decant) culture medium from cells.
 - **Note:** If the culture medium contained phenol red or other reagents that possibly interfere with subsequent protein analysis, wash cells once in wash buffer (e.g., PBS).
- 2. Add the appropriate amount of N-PER Reagent to the plate or to each plate well (Table 1). Incubate on ice for 5 minutes.



Table 1. Suggested volume of Thermo Scientific N-PER Reagent to use for different sizes of standard culture plates.

Description of the control of the co	
Plate Size/Surface Area	N-PER Reagent Volume
100mm	500-1000μL
60mm	250-500μL
6-well plate*	200-400μL per well
24-well plate	100-200μL per well
96-well plate	50-100μL per well

^{*}Primary neuronal cells grown in six-well plates typically contain 10⁶ cells in each well and yield ~0.3mg of total protein per well.

- 3. Scrape the plate surface using a cell scraper to lift the cells. Collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at $10,000 \times g$ for 10 minutes to pellet the cell debris.
- 4. Transfer supernatant to a new tube for analysis.

Related Thermo Scientific Products

78420	Halt™ Phosphatase Inhibitor Cocktail (100X), 1mL
87785	Halt Protease Inhibitor Cocktail, EDTA-free (100X), 1mL
87786	Halt Protease Inhibitor Cocktail Kit, 1mL
78440	Halt Protease and Phosphatase Inhibitor Cocktail (100X), 1mL
78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), 1mL
88660	Pierce® Protease Inhibitor Tablets, 30 tablets
88661	Pierce Protease Inhibitor Tablets, EDTA-free, 30 tablets
88662	Pierce Phosphatase Inhibitor Tablets, 20 tablets
88663	Pierce Protease and Phosphatase Inhibitor Tablets, 20 tablets
88664	Pierce Protease and Phosphatase Inhibitor Tablets, EDTA-free, 20 tablets
87791	Pierce Tissue Strainers, 250μm, 50 each
87793	Syn-PER TM Synaptic Protein Extraction Reagent, 100mL
87790	Subcellular Protein Fractionation Kit for Tissues
23225	Pierce BCA Protein Assay Kit

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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