INSTRUCTIONS

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

2-D Sample Prep for Nuclear Proteins

89863

1431.1

Number	Description
89863	**2-D Sample Prep for Nuclear Proteins,** contains sufficient components to prepare and process 25 nuclear extracts for 2-D analysis

Kit Contents:

NE-PER® Nuclear and Cytoplasmic Extraction Reagents, contains sufficient lysis reagents for extracting 25 cell pellet fractions with a packed cell weight of 20-40 mg each; NE-PER® Reagents include the following components:

- **Cytoplasmic Extraction Reagent I (CER I)**, 5 ml, store at 4°C
- **Cytoplasmic Extraction Reagent II (CER II)**, 275 µl, store at 4°C
- **Nuclear Extraction Reagent (NER)**, 2.5 ml, store at 4°C

2-D Sample Buffer for Nuclear Proteins, contains sufficient reagents for the preparation of 30 ml of 2-D sample buffer containing 7 M Urea, 2 M Thiourea and 4% CHAPS; the buffer is prepared from the following components:

- **2-D Sample Buffer for Nuclear Proteins Component A**, 18 ml, store at 4°C
- **2-D Sample Buffer for Nuclear Proteins Component B**, 16.5 g, store at ambient temperature

Protein Desalting Spin Columns, 25 columns, each column containing ~700 µl of desalting slurry buffered in 10 mM Tris•HCl, pH 7.5 containing 0.02% sodium azide; store at 4°C

Storage: Upon receipt store 2-D Sample Buffer Component B at ambient temperature and all other components at 4°C. Product is shipped at ambient temperature.

Table of Contents

Introduction....2
Important Product Information....2
Additional Materials Required....2
Protocol for the 2-D Sample Prep for Nuclear Proteins....2
A. Preparation of Nuclear Extract....2
B. Preparation of 2-D Sample Buffer for Nuclear Proteins (7 M Urea, 2 M Thiourea, 4% CHAPS)....3
C. Preparation and Equilibration of Protein Desalting Spin Columns....3
D. Sample Loading and Desalting....4
E. Preparation of Desalted Nuclear Sample for IPG Strip Rehydration....4
Related Pierce Products....4
References....4

Warranty: Pierce products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sale for products used, handled and stored according to Pierce instructions. Pierce's sole liability for the product is limited to replacement of the product or refund of the purchase price. Pierce products are supplied for laboratory or manufacturing applications only. They are not intended for medicinal, diagnostic or therapeutic use. Pierce products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Pierce Biotechnology. Pierce strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce product, please contact Pierce or your local distributor.

Introduction

The 2-D Sample Prep for Nuclear Proteins provides a complete set of components for the preparation of nuclear extract from cultured mammalian cells and tissues, removal of salt from the prepared extract, and exchange of the nuclear proteins into buffer that is compatible for 2-D gel electrophoresis. Nuclear extracts are prepared using NE-PER® Nuclear and Cytoplasmic Extraction Reagents. Protein desalting spin columns are subsequently used to remove >98% of the salt contaminants to prevent interference with isoelectric focusing. The desalting columns are pre-equilibrated in 2-D Sample Buffer so recovered sample can be immediately applied to an Immobilized pH Gradient (IPG) strip in its entirety, effectively increasing the allowable sample load volume.

The 2-D Sample Prep for Nuclear Proteins was developed for streamlining nuclear protein extraction with 2-D sample preparation. With this sample prep kit, nuclear proteins with a MW >7K are desalted and recovered with ≥90% efficiency from HeLa, NIH 3T3 and C6 cell lines as well as mammalian tissue. The 2-D Sample Buffer contains multiple chaotropes that increase the solubility of nuclear proteins, resulting in minimal sample loss and high-resolution 2-D gels.[1] Multiple samples may be processed simultaneously using a rapid and efficient protocol to quickly obtain 2-D gels of nuclear proteins.

Important Product Information

- Although this sample prep kit provides reagents for the extraction of both cytoplasmic and nuclear proteins, it is intended for purification of the nuclear extract only. The cytoplasmic extract obtained using this procedure does not contain contaminants incompatible with 2-D gel electrophoresis and therefore does not require processing before 2-D gel analysis. Please note that this sample prep kit does **NOT** provide enough 2-D sample buffer for applications with the cytoplasmic extract.
- For optimal results, include protease inhibitors (e.g., Product No. 78415) in the CER I and NER.
- The NE-PER® Reagents in this sample prep kit provide for 25 protein extractions from packed cell pastes of approximately 20-40 mg each (refer to Table 1). Using cell weights >40 mg requires larger volumes of NE-PER® Reagents and will, therefore, decrease the number of applications obtainable.

Additional Materials Required

- Variable-speed bench-top microcentrifuge
- 1.5-2.0 ml microcentrifuge collection tubes
- Reducing agent such as DTT (Product No. 20290)
- Carrier Ampholytes
- Bromophenol Blue (optional)

Protocol for the 2-D Sample Prep for Nuclear Proteins

A. Preparation of Nuclear Extract

Note: Perform centrifugation procedures at 4°C. Keep cell samples, extracts and NE-PER® Reagents on ice.

Table 1. Reagent requirements for preparing one nuclear protein sample.

Weight of cell paste	CER I	CER II	NER
20-40 mg	200 µl	11 µl	100 µl

1. Isolate 20-40 mg of cells (refer to Table 1) by centrifugation in a 1.5 ml microcentrifuge tube at 500 x *g* for 2 minutes.

   Note: If using tissue samples, weigh ~20 mg of the tissue and cut it into small pieces. Add 200 µl of ice-cold CER I, and homogenize in a tissue homogenizer. Proceed to step A.4.

2. Using a pipette, carefully remove and discard the supernatant, leaving the cell pellet as dry as possible.
3. Add 200 µl of ice-cold CER I to the cell pellet.
4. Vortex vigorously on the highest setting for 15 seconds to fully resuspend the cell pellet. Incubate tube on ice for 10 minutes.

5. Add 11 µl of ice-cold CER II to the tube.
6. Vortex tube for 5 seconds on the highest setting. Incubate tube on ice for 1 minute.
7. Vortex tube for 5 seconds on the highest setting. Centrifuge tube at 16,000 x *g* for 5 minutes.
8. Immediately transfer the supernatant (cytoplasmic extract) fraction to a new pre-chilled tube. Place this tube on ice.
9. Resuspend the pellet (nuclear fraction) produced in step A.7 in 100 µl of ice-cold NER.
10. Vortex tube on the highest setting for 15 seconds. Return the sample to ice and continue to vortex for 15 seconds every 10 minutes, for a total of 40 minutes.
11. Centrifuge tube at 16,000 x *g* for 10 minutes.
12. Immediately transfer the supernatant (nuclear extract) fraction to a new pre-chilled tube, and place it on ice.
13. Store all extracts at -70°C until use.

B. Preparation of 2-D Sample Buffer for Nuclear Proteins (7 M Urea, 2 M Thiourea, 4% CHAPS)

Note: The following procedure prepares 1.2 ml of 2-D Sample Buffer sufficient to equilibrate one column and adjust the volume of the sample before and after desalting. Increase volumes accordingly for multiple samples. Use Sample Buffer within 4 hours of preparation.

1. Mix the bottle containing 2-D Sample Buffer Component B (dry component) by inverting several times to obtain a homogeneous powder.
2. Weigh 0.66 g of 2-D Sample Buffer Component B into a 1.5 ml microcentrifuge tube.
3. Add 720 µl of 2-D Sample Buffer Component A (liquid component) to the tube containing 0.66 g of 2-D Sample Buffer Component B.
4. Thoroughly mix the two components until a homogeneous suspension is obtained.
5. Maintain the prepared 2-D Sample Buffer at ambient temperature until use.

C. Preparation and Equilibration of Protein Desalting Spin Columns

1. Invert column several times to suspend slurry.
2. Twist off bottom closure and loosen cap.

   Note: Do not snap off bottom closure. To remove closure, twist it slightly in one direction followed by the other direction.
3. Place column in 1.5-2.0 ml microcentrifuge collection tube.
4. Centrifuge column at 1,500 x *g* for 1 minute to remove excess liquid.
5. Blot bottom of column on a paper towel to remove any excess trapped liquid.
6. Empty the collection tube.
7. Return column to the collection tube.

   Note: Place a mark on the side of the column where the compacted resin is slanted upward. Place column in microcentrifuge with the mark facing outward in all subsequent centrifugation steps.
8. Carefully add 300 µl of 2-D Sample Buffer (prepared in Part B) to the top of the column. Do not mix.
9. Centrifuge column at 1,500 x *g* for 1 minute to remove excess liquid.
10. Discard buffer from the collection tube and return column to the collection tube.
11. Repeat steps C.8-C.10.
12. Carefully add 300 µl of 2-D Sample Buffer to the top of the column.
13. Centrifuge at 1,500 x *g* for 2 minutes to remove excess liquid.
14. Discard collection tube and transfer column to a new collection tube.

D. Sample Loading and Desalting

Note: For optimal results, perform a protein assay on the nuclear extract to determine its protein concentration before proceeding with desalting. (Refer to Related Pierce Products section.)

Note: The amount of nuclear protein required for 2-D gel analysis is dependent upon the size of the IPG strip used, the gel staining method and the total number of gels that will be processed with the sample. Consult with the IPG strip manufacturer for recommendations concerning the amount of protein to apply.

1. Mix desired amount of nuclear extract with 2-D Sample Buffer to a final volume of 50 µl.
2. Apply the 50 µl of prepared sample to the center of the compacted resin bed. Do not disturb the resin or allow sample to flow around the resin bed.
3. Centrifuge column at 1,500 x *g* for 2 minutes. The collected volume (eluate) is ~60 µl and contains the sample desalted and exchanged into 2-D Sample Buffer.
4. Discard desalting column after use.

E. Preparation of Desalted Nuclear Sample for IPG Strip Rehydration

Note: Consult with the IPG strip manufacturer for recommended total sample volume required for desired IPG strip rehydration and for recommended amounts of reducing agent and carrier ampholytes. This sample prep provides sufficient 2-D Sample Buffer for 25 samples, each applied to an 18 cm IPG strip. Fewer than 25 applications will be obtained if larger IPG strips are used.

1. Add 2-D Sample Buffer (prepared in Part B) to desalted nuclear sample (prepared in Part D) to predetermined final sample volume (refer to Note).
2. Add reducing agent and carrier ampholytes.
3. (Optional) Add a trace amount of Bromophenol Blue to sample.
4. Follow manufacturer's instructions for rehydration of IPG strip.

Related Pierce Products

20290	**DTT,** 5 g
23225	**BCA Protein Assay Reagent Kit,** sufficient reagents to perform 500 standard tube assays or 5,000 microplate assays
26659	**2-D Protein Molecular Weight Marker Mix,** 500 µl, sufficient reagent for up to 1,000 mini-gels stained with silver stain
26671	**ColorMeRanger™ Unstained Protein Molecular Weight Marker Mix,** 48-96 doses
24612	**GelCode® SilverSNAP® Silver Stain Kit II**, sufficient to stain 20 mini gels
78415	**Halt™ Protease Inhibitor Cocktail, EDTA-Free,** 1 ml

References

1. Rabilloud, T., *et al*. (1997). Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis*. **18:**307-316.

©Pierce Biotechnology, Inc., 11/2003. Printed in the USA.