INSTRUCTIONS

PIERCE
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2-D Sample Prep for Soluble or Insoluble Proteins

89865 89866

1432.2

Number	Description
89865	**2-D Sample Prep for Soluble Proteins,** contains sufficient components to process 25 protein samples

Kit Contents:

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

2-D Sample Buffer for Soluble Proteins, contains sufficient reagents for the preparation of 30 ml of 2-D Sample Buffer containing 8 M urea and 4% CHAPS; the buffer is prepared from the following components:

2-D Sample Buffer for Soluble Proteins Component A, 19.5 ml, store at 4°C

2-D Sample Buffer for Soluble Proteins Component B, 15 g, store at ambient temperature

89866	**2-D Sample Prep for Insoluble Proteins,** contains sufficient components to process 25 low-solubility protein samples

Kit Contents:

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

2-D Sample Buffer for Insoluble 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 Insoluble Proteins Component A, 18 ml, store at 4°C

2-D Sample Buffer for Insoluble Proteins Component B, 16.5 g, store at ambient temperature

Storage: Upon receipt store 2-D Sample Buffer Component B (89865B or 89866B) at ambient temperature and all other components at 4°C. Product is shipped at ambient temperature.

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Introduction

The 2-D Sample Prep for Soluble Proteins and the 2-D Sample Prep for Insoluble Proteins rapidly remove salts, buffers and other small ionic contaminants from cultured cell extracts and tissues. These sample preps not only remove the contaminants, but they also exchange the proteins into a buffer that is compatible for 2-D gel electrophoresis. The 2-D Sample Buffer for Soluble Proteins (8 M urea, 4% CHAPS) is for whole cell extracts and cytosolic preparations in which the proteins of interest are predominantly hydrophilic. 2-D Sample Buffer for Insoluble Proteins (7 M urea, 2 M thiourea, 4% CHAPS) is for proteins that are more difficult to solubilize such as large and/or hydrophobic proteins in fractionated cell extracts, proteins that tend to aggregate, and nuclear proteins.[1]

Charged species interfere with 2-D gel analysis by prolonging isoelectric focusing and distorting the gel at the pH extremes. These sample prep kits use Protein Desalting Spin Columns to remove >98% of small charged contaminants in less than 10 minutes. The desalting columns are pre-equilibrated in 2-D Sample Buffer before use to maintain protein solubility and improve recovery. Desalted proteins with a MW >7 K are recovered with ~80% efficiency from whole cell lysates of HeLa and NIH 3T3 cell lines and ~90% efficiency from rat liver homogenates prepared with T-PER® Tissue Protein Extraction Reagent. Because proteins are recovered in 2-D Sample Buffer, they can be immediately applied to an Immobilized pH Gradient (IPG) strip in their entirety, effectively increasing the allowable sample load volume. Furthermore, multiple samples can be processed simultaneously.

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 Soluble or Insoluble Proteins

A. Preparation of 2-D Sample Buffer

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

- **For Soluble Proteins (8 M urea and 4% CHAPS, Component No. 89865A and No. 89865B):**

1. Mix the bottle containing 2-D Sample Buffer Component B (dry component) by inverting several times to obtain a homogeneous powder.
2. Weigh 0.6 g of 2-D Sample Buffer Component B into a 1.5 ml microcentrifuge tube.
3. Add 780 µl of 2-D Sample Buffer Component A (liquid component) to the tube containing 0.6 g of 2-D Sample Buffer Component B.
4. Thoroughly mix the two components until a homogeneous suspension results.
5. Maintain the prepared 2-D Sample Buffer at ambient temperature until use.

- **For Insoluble Proteins (7 M urea, 2 M thiourea and 4% CHAPS, Component No. 89866A and No. 89866B):**

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 results.
5. Maintain the prepared 2-D Sample Buffer at ambient temperature until use.

B. 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 A) 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 B.8-B.10.
12. Carefully add 300 µl of 2-D Sample Buffer to the top of the column.
13. Centrifuge column at 1,500 x *g* for 2 minutes to remove excess liquid.
14. Discard collection tube and transfer column to a new collection tube.

C. Sample Loading and Desalting

Note: For optimal results, perform a protein assay on the sample before desalting (refer to Related Pierce Products section). The amount of protein required for 2-D 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 protein 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 Protein Sample for IPG Strip Rehydration

Note: Consult with the IPG strip manufacturer for recommended total sample volume required for desired IPG strip rehydration as well as 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 A) to desalted protein sample (prepared in Part C) to predetermined final sample volume for IPG strip (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® Stain Kit II**, sufficient to stain 20 mini gels
78415	**Halt™ Protease Inhibitor Cocktail, EDTA-Free,** 1 ml
78510	**T-PER® Tissue Protein Extraction Reagent,** 500 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.