INSTRUCTIONS

# Protein Desalting Spin Columns

89849 89862

PIERCE
a brand of PERBIO
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

1377w

| Number | Description |
|---|---|
| **89849** | **Protein Desalting Spin Columns**, 25 columns per package, each column containing ~700 µl of desalting resin buffered in 10 mM Tris, pH 7.5 containing 0.02% sodium azide |
| **89862** | **Protein Desalting Spin Columns**, 50 columns per package, each column containing ~700 µl of desalting resin buffered in 10 mM Tris, pH 7.5 containing 0.02% sodium azide |
| | Storage: Upon arrival store at +4°C. Product shipped at ambient temperature. |
| | *This product is guaranteed for one year from the date of purchase when handled and stored properly.* |

## Introduction

Pierce Protein Desalting Spin Columns are designed to desalt or exchange buffer of protein samples with volumes from 30 to 120 µl. Protein Desalting Spin Columns have exceptional desalting characteristics with ≥ 95% retention of salts and small molecules while providing excellent recovery of proteins greater than 7,000 MW. Multiple samples can be processed in less than 5 minutes. No cumbersome column preparation steps are required.

## Procedure for Protein Desalting Spin Column

**Additional Materials Required**

- Variable-speed bench-top microcentrifuge
- 1.5-2.0 ml microcentrifuge collection tubes

**Sample Loading Guidelines**

Sample purity and yield obtained after desalting is largely dependent on the sample loading volume. For optimal results, load sample volumes from 30 to 120 µl. If sample is of high ionic strength, such as 0.5-1M $CaCl_2$, reduce maximim sample volume to 75 µl.

**A. Protein Desalting Spin Column Preparation**

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

   **Note**: Do not snap off bottom. To remove, twist slightly in one direction followed by the other direction.

3. Place column in 1.5-2.0 ml microcentrifuge collection tube.
4. Centrifuge 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.

**B. Sample Loading**

1. Place column in a fresh collection tube, remove cap and apply 30-120 µl of sample to the center of the compacted resin bed. *Be careful not to disturb the resin or to allow sample to flow around the resin bed.*
2. (Optional) To improve recovery percentage of low molecular weight proteins or for small sample volumes, add 20-40 µl of 10 mM Tris Buffer, pH 7.5, to top of resin. Do not exceed a total sample plus stacker volume of 120 µl.
3. Centrifuge at 1,500 x *g* for 2 minutes. The desalted sample is collected in collection tube.
4. Discard desalting column after use.

## Procedure for Buffer Exchange

**Additional Materials Required**

- Variable-speed bench-top microcentrifuge
- 1.5-2.0 ml microcentrifuge collection tubes
- Equilibration buffer in which protein to be exchanged

**A. Protein Desalting Spin Column Preparation**

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

   **Note**: Do not snap off bottom. To remove, twist slightly in one direction followed by the other direction.

3. Place column in 1.5-2.0 ml microcentrifuge collection tube.
4. Centrifuge at 1,500 x *g* for 1 minute to remove excess liquid.
5. Add 400 µl of equilibration buffer to the top of column.
6. Spin the column at 1,500 x *g* for 1 minute to remove excess liquid.
7. Repeat steps 4 and 5 two to three additional times, discarding buffer from the collection tube.

**B. Sample Loading**

1. Place column in a fresh collection tube, remove cap, and apply 30-120 µl of sample to the center of the compacted resin bed. *Be careful not to disturb the resin or to allow sample to flow around the resin bed.*
2. (Optional) To improve recovery percentage of low molecular weight proteins or for small sample volumes, add 20-40 µl of Equilibration Buffer to top of resin. Do not exceed a total sample plus stacker volume of 120 µl.
3. Centrifuge at 1,500 x *g* for 2 minutes. The desalted sample is collected in collection tube.
4. Discard desalting column after use.

## Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| Sample or buffer does not flow through resin | Centrifugation problem | Ensure that centrifuge is in proper working condition<br>Ensure bottom closure is removed |
| Contamination in sample | Improper sample loading | Load sample directly in center of the resin bed; tip touch to expel all sample; do not "blow out" the tips<br>Avoid contact with sides of the column |
| | High molecular weight contaminate | If contaminant is 700-2,000 MW, lower sample volume applied to the column |
| | Centrifugation problem | Do not exceed recommended centrifuge times or speeds |
| Low yield | Centrifugation problem | Apply 20-40 µl buffer overlay on top of sample before centrifugation<br>Increase sample concentration<br>Increase load volume |

## Related Pierce Products

| Number | Description |
|---|---|
| **43240** | **D-Salt™ Polyacrylamide Plastic Desalting Columns**, 5 x 5 ml |
| **69550** | **Slide-A-Lyzer® MINI Dialysis Unit 3.5K MWCO**, 10-100 µl Capacity, 50/pkg |

D-Salt™ is a trademark of Pierce Biotechnology, Inc.
The Slide-A-Lyzer® MINI Dialysis Unit is protected by U.S. Patent # 6,039,871.