Introduction
The Zeba Desalt Spin Columns contain a high-performance resin that offers exceptional desalting or buffer-exchange for protein samples. Samples containing as low as 20 µg of protein/ml can be processed with unsurpassed protein recovery and ≥ 95% retention of salts and other small molecules (< 1,000 Da). These columns require no chromatography system or cumbersome preparation or equilibration. The spin-column method eliminates waiting for samples to emerge by gravity flow and the subsequent monitoring of fractions for protein recovery, enabling the processing of multiple samples in ~8 minutes.

Procedure for Protein Desalting
A. Additional Materials Required
   • Variable-speed centrifuge
   • 15 ml conical collection tubes or equivalent for the 2 ml and 5 ml spin columns
   • 50 ml conical collection tubes for the 10 ml spin columns

B. Spin Column Preparation and Sample Loading
1. Twist off the column’s bottom closure and loosen cap. Place column in a collection tube.
2. Centrifuge column at 1,000 × g for 2 minutes to remove storage solution. When using fixed-angle rotors, place a mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.
   Note: Resin will appear compacted after centrifugation.
3. Place column in a new collection tube, remove cap and slowly apply sample to the center of the compact resin bed.
   Note: See Table 1 for recommended sample volumes for each column size.
4. (Optional) To ensure maximal protein recovery from low-volume samples, apply a stacker of ultrapure water or buffer to the resin bed after sample has fully absorbed.
   Note: See Table 1 for sample volumes for which a stacker is recommended.
5. Centrifuge column at 1,000 × g for 2 minutes to collect desalted sample. Discard column after use.
Procedure for Buffer Exchange

A. Additional Materials Required
   - Variable-speed centrifuge
   - 15 ml conical collection tubes or equivalent for the 2 ml and 5 ml spin columns
   - 50 ml conical collection tubes for the 10 ml spin columns
   - Buffer for exchange

B. Protein Desalting Spin Column Preparation
   1. Twist off the column’s bottom closure and loosen cap. Place column in a collection tube.
   2. Centrifuge column at $1,000 \times g$ for 2 minutes to remove storage solution. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.
      Note: Resin will appear compacted after centrifugation.
   3. Add buffer to the column.
      - For 2 ml columns use 1 ml of buffer.
      - For 5 ml columns use 2.5 ml of buffer.
      - For 10 ml columns use 5 ml of buffer.
   4. Centrifuge at $1,000 \times g$ for 2 minutes to remove buffer.
   5. Repeat steps 3 and 4 two or three additional times, discarding buffer from the collection tube.

C. Sample Loading
   1. Place column in a new collection tube, remove cap and slowly apply sample to the center of the compact resin bed.
      Note: See Table 1 for recommended sample volumes for each column size.
   2. (Optional) To ensure maximal protein recovery from low-volume samples, apply a stacker of ultrapure water or buffer to the resin bed after the sample has fully absorbed.
      Note: See Table 1 for sample volumes for which a stacker is recommended.
   3. Centrifuge at $1,000 \times g$ for 2 minutes to collect the sample. Discard column after use.

<table>
<thead>
<tr>
<th>Column</th>
<th>Sample Volume</th>
<th>Optional Stacker Volume*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ml</td>
<td>200-700 µl</td>
<td>40 µl stacker for samples &lt; 350 µl</td>
</tr>
<tr>
<td>5 ml</td>
<td>500-2,000 µl</td>
<td>100 µl stacker for samples &lt; 750 µl</td>
</tr>
<tr>
<td>10 ml</td>
<td>700-4,000 µl</td>
<td>200 µl stacker for samples &lt; 1,500 µl</td>
</tr>
</tbody>
</table>

* To ensure maximal protein recovery, apply a stacker to the resin after sample has entered the resin bed.
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
</table>
| Sample or buffer does not flow through resin | Centrifugation problem                             | Ensure that centrifuge is in proper working condition  
|                                               |                                                     | Ensure bottom closure is removed                   |
| Sample contamination                         | Improper sample loading                             | Apply sample directly to center of the resin bed; touch tip to resin to expel all sample  
|                                               |                                                     | Avoid contact with sides of column                  |
|                                               | Improper centrifugation                             | For fixed-angle rotors, place column in the same orientation each time  
|                                               |                                                     | Do not exceed recommended centrifugation speed or time |
| Low yield                                    | No stacker used                                     | Apply a stacker above sample                        |
| Unstable protein                             |                                                     | Equilibrate column in a suitable buffer             |

## Related Products

- **23225**  **Pierce BCA Protein Assay Kit**, for 20-2,000 μg/ml samples
- **23235**  **Pierce Micro BCA Protein Assay Kit**, for 0.5-20 μg/ml samples
- **89877**  **Zeba Micro Desalt Spin Columns**, 25 each, for 2-12 μl samples
- **89879**  **Zeba Micro Spin Columns (Empty)**, 50/pkg., 100 μl gel-bed capacity
- **89882**  **Zeba Desalt Spin Columns, 0.5 ml**, 25 each, for 30-130 μl samples

U.S. patent pending on Zeba™ Column Technology.

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Current versions of product instructions are available at [www.thermo.com/pierce](http://www.thermo.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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