

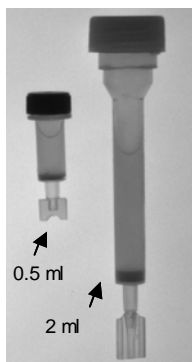
## Protein Desalting/Buffer Exchange Protocol

All Solulink protein modification protocols require that proteins first be desalted prior to modification. Desalting removes small, interfering amine contaminants from the sample while exchanging the proteins into specially optimized reaction buffers. Solulink recommends the use of Zeba™ Desalt Spin Columns (Pierce Chemical Inc.) for this purpose. Zeba columns are available in 2 sizes. The 0.5 ml spin columns are capable of desalting volumes up to 130 µl. The 2 ml size can process sample volumes up to 700 µl (see Figure 1 below). Choose the size column for the appropriate sample volume.

**NOTE**-Zeba™ is a registered trademark of Pierce Chemical

**IMPORTANT**-Always use 1X Modification buffer pH 7.4 to desalt proteins **prior** to modification and 1X Modification buffer pH 6.0 to desalt proteins **after** modification. A slightly basic solution (pH 7.4) is required for optimal modification of proteins and a slightly acidic solution (pH 6.0) is required for optimal conjugation of modified proteins.

**NOTE**- larger 2 ml Zeba™ Spin Column do not fit into high-speed microcentrifuges that hold standard 1.5 ml tubes. The larger Zeba™ spin column requires a tabletop or floor-based centrifuge capable of spinning 15 ml conical tubes.



**Figure 1.** Zeba™ Desalt Spin Columns (0.5 and 2 ml) used to desalt proteins before and after biotinylation.

### Materials required

Zeba Desalt Spin Columns (0.5 ml (Cat # 89882) or 2 ml (Cat. # 89889) available from Pierce Chemical

Microcentrifuge (holds 1.5 ml tubes)

Table Top Centrifuge (holds 15 ml conical tubes)

P-100 and 1000 pipettes

## Zeba™ Desalt/Buffer Exchange Protocol

### 0.5 ml Zeba™ Spin Column

#### Column Preparation (130 µl max. sample processing volume)

1. Remove spin column's bottom closure and loosen the top cap (do not remove cap).
2. Place spin column in a 1.5 ml microcentrifuge collection tube.

3. Centrifuge at 1500xg for 1 minute to remove storage solution.
4. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in the microfuge with the mark facing outward in all subsequent centrifugation steps.
5. Add 300  $\mu$ l of 1x Modification buffer (pH 7.4) to the top of the resin bed and centrifuge at 1500xg for 1 minute, discard flow-through from collection tube.
6. Repeat step 4 and 5 two additional times, discarding buffer from the collection tube each time.
7. Column is now ready for sample loading.

### **Protein Sample Loading**

1. Place the equilibrated spin column into a new 1.5 ml collection tube, remove cap and slowly apply up to a 130  $\mu$ l sample volume to the center of the compact resin bed.

**NOTE-** for sample volumes less than 70  $\mu$ l apply a 15  $\mu$ l buffer (stacker) to the top of the resin bed after the sample has fully absorbed to ensure maximal protein recovery. Avoid contact with the sides of the column when loading.

2. Centrifuge at 1500xg for 2 minutes to collect desalted sample.
3. Discard column after use.
4. Protein sample is now desalted/buffer exchanged and ready for use.

## **2 ml Zeba™ Spin Column**

### **Column Preparation (700 $\mu$ l max. sample processing volume)**

1. Twist off the column's bottom closure and loosen the top cap. Place column in a 15 ml conical collection tube.
2. Centrifuge column at 1000xg for 2 minute to remove storage solution.
3. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in the centrifuge with the mark facing outward in all subsequent centrifugation steps.
4. Add 1ml of 1x Modification buffer (pH 7.4) to the top of the resin bed.
5. Centrifuge at 1000xg for 2 minute to remove buffer.
6. Repeat step 4 and 5 two or three additional times, discarding buffer from the collection tube each time.
7. Column is now ready for sample loading.

### **Protein Sample Loading**

1. Place spin column into a new 15 ml conical collection tube, remove cap and slowly and apply a sample volume (240-700  $\mu$ l) to the center of the compact resin bed.

**NOTE-** for sample volumes less than 350  $\mu$ l apply additional buffer (stacker) to the top of the resin bed (i.e. 40  $\mu$ l) after the sample has fully absorbed to ensure maximal protein recovery. Avoid contact with the sides of the column when loading.

2. Centrifuge at 1000xg for 2 minutes to collect desalted sample.
3. Discard column after use and retain the desalted protein in the 15 ml conical tube.
4. Protein sample is now desalted/buffer exchanged and ready for further use.