USER GUIDE

Protein Buffer Exchange and Desalting Protocol

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Protein modification protocols require a buffer exchange and desalting of proteins. Desalting removes small, interfering amine contaminants from the sample while exchanging the proteins into specially optimized reaction buffers. Thermo Scientific™ Zeba™ Desalting Columns are recommended for this purpose and can desalt volumes up to 130 µl.

Modification Buffer, pH 8.0 is recommended to desalt proteins prior to modification and Conjugation Buffer, pH 6.0 to desalt proteins after modification. A slightly basic solution (pH 8.0) is required for optimal modification of proteins and a slightly acidic solution (pH 6.0) is required for optimal conjugation of modified proteins.

A. Column Preparation

1. Remove the column's bottom closure, Figure 1. Loosen the cap (do not remove).
2. Place spin column in a 1.5 ml microcentrifuge collection tube.
3. Centrifuge at 1,500 x g 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 microcentrifuge with the mark facing outward in all subsequent centrifugation steps.
5. Add 300 µl of 1X Modification buffer (pH 8.0) or appropriate buffer to the top of the resin bed and centrifuge at 1,500 x g for 1 minute. Then discard flow-through from collection tube.
6. Repeat steps 4 and 5 two additional times, discarding buffer from the collection tube each time.
7. Column is now ready for sample loading.

B. 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 µl sample volume to the center of the compact resin bed.

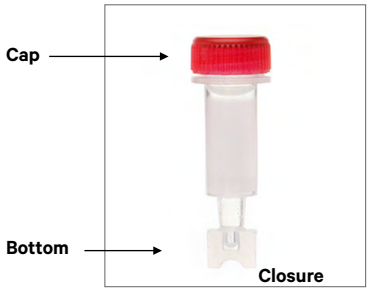

Figure 1. Zeba Desalting Column

Note: for sample volumes less than 70 µl, apply 15 µl 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 1,500 x g for 2 minutes to collect desalted sample.
3. Discard column after use.
4. Protein sample is now desalted & buffer exchanged and ready for use.

Materials Required

Reagents	Equipment
Zeba Desalting Columns	Variable-speed bench-top microcenttrifuge
Modification Buffer*	1.5 ml microcentrifuge tubes
Conjugation Buffer*	

*Depending on desired final buffer

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