

Protein Buffer Exchange and Desalting Protocol

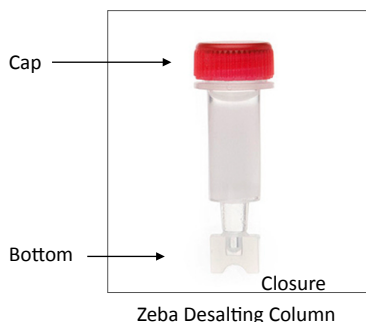
Protein modification protocols require a buffer exchange and desalting of proteins. Desalting removes small, interfering amine contaminants from the sample while exchanging the proteins into specially optimized reaction buffers. Zeba Desalting Columns are recommended for this purpose and can desalt volumes 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 1500 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 1500 x g for 1 minute. Then 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.

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.
 - a. 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 1500 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 microcentrifuge
Modification Buffer*	1.5 mL microcentrifuge tubes
Conjugation Buffer*	

*Depending on desired final buffer