FOR RESEARCH USE ONLY!

EZ-Desalt™ Spin Desalting Columns

rev.10/13

Store at 4°C. Do not freeze.

Cat. No.

6564-25 **25 EZ-Desalt™ Spin Desalting Columns of Pre-swollen Sephadex G-25** (0.5 ml in PBS with 0.1% sodium azide)

I. Description:

AMSBIO's EZ-Desalt™ Spin Desalting Column offers a convenient option to quickly desalt, buffer-exchange, or clean-up protein samples in solution. Each of the disposable, ready-to-use, spin desalting column contains 0.5 ml pre-swollen Sephadex G-25 beads, that efficiently separates proteins (or other high molecular weight components) from small molecules (salt and other small molecules such as LMW dyes etc.). Large molecules are too big to enter the pores of the beads and are excluded from Sephadex matrix and are eluted immediately after the void volume of the column, whereas small molecules are retained by the resin, with the cut-off size of about 7 kDa. The whole process takes few min. and achieves an excellent recovery of proteins (>90%), and efficient removal of the salt or unwanted small molecules. The column can be used in either a swing bucket or fixed angle rotors for 2.0 ml and 1.5 ml centrifuge tubes at a centrifugation force of 1,500 x g. The volume of samples is within the range of 30 to 130 ul.

II. Applications:

- Removal of undesired small molecules in protein purification (such as Imidazole in His tag affinity purified proteins)
- Buffer exchange for proteins into a different buffer compatible for downstream applications
- Removal of free biotin molecules following a protein biotinylation reaction
- Removal of free radioactive labels from radiolabeled protein targets such as ^{32}P-GTPase, or ^{32}P phosphorylated protein
- Clean-up of protein Click-Chemistry reaction samples by removal of free small molecules
- Removal of small molecules interfering with downstream application, such as LMW enzyme inhibitor in an enzymatic reaction
- Removal of free fluorescent dyes after labeling of proteins

III. User Supplied Reagents and Equipment:

- Exchange Buffer: If samples need to be in a buffer other than EZ-Desalt™ Spin Desalting Column storage buffer (PBS with 0.1% sodium azide), the column has to be equilibrated with the desired buffer, as described in the Procedure under Section A, before loading your sample.
- Microcentrifuge tubes: Regular 1.5 ml and 2.0 ml microcentrifuge tubes.
- A centrifuge with a fixed angle or a swing bucket rotor: for 1.5 ml or 2.0 ml microcentrifuge tubes.

IV. Procedure for Desalting, Buffer Exchange or Sample Clean-up

A. Column Preparation and Equilibration:

1. Snap off the bottom closure and slightly loosen the cap. Place the column in a 2.0 ml microcentrifuge tube, centrifuge at 1,500 x g for 1 min. to remove the storage buffer.

   Note: with a fixed angle rotor, the resin tends to slant upward toward the center of rotor; mark that side of microcentrifuge tube.
2. Carefully apply 350 µl of desired buffer to the center of column, place column back to centrifuge tube with the marked side facing outward. Centrifuge for 1,500 x g for 1 min., and remove buffer.
3. Repeat step 2 three times, discarding the buffer in centrifuge tubes each time. Column is ready for the samples.

B. Sample Application and Centrifugation:

1. Place the column in a new centrifuge tube (1.5 or 2.0 ml), after making sure that there is no residual solution at the column bottom (wipe it, if any). Carefully apply sample (30 to 130 µl) to the center of the column, and centrifuge at 1,500 x g for 2 min.
2. Collect solution in the centrifuge tube – this is the final protein sample.

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