Waters™

Sep-Pak™ SEC Desalting Cartridges, 1cc 5K MWCO Care and Use Manual

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I. INTRODUCTION

The Sep-Pak SEC Desalting Cartridge, 1cc 5K MWCO (Molecular Weight Cut-Off) is based on a syringe barrel configuration that is designed for gravity-assisted flow. The product consists of a 1cc flangeless cartridge containing a storage solution of 20% ethanol/ 80% water and it is supplied capped on the top and bottom to retain the storage solution within. Using these devices, scientists will be able to purify, desalt and buffer exchange proteins above MW of 5,000 using a manual workflow or an automated workflow on the Andrew+™ Pipetting Robot. The cartridges are QC tested to obtain ≥80% protein recovery to ensure reproducibility.

The sorbent used in the Sep-Pak SEC Desalting Cartridge is a cross-linked dextran with a pH range from 2 to 13. The Sep-Pak SEC Desalting Cartridge is used with the Waters PeptideWorks™ Tryptic Protein Digestion Kits for the removal of denaturant, reduction and alkylation reagents prior to digestion, refer to the PeptideWorks Care & Use Manual. The cartridges require the 1cc Cartridge Stand (p/n 186010128) for a manual workflow for proper use to hold cartridges and process samples for gravity-assisted flow.

Sep-Pak SEC Desalting Cartridges are developed for general laboratory research use only and are not intended for use for *in vitro* diagnostics.

a. Contents Included

Sep-Pak SEC Desalting Cartridges are available in different configurations. See Table 1 for a description of the contents for each available part number.

Table 1. Sep-Pak SEC Desalting Cartridges Descriptions by Part Number

Description	Part Number
Sep-Pak SEC Desalting Cartridge, 1cc Start-Up Kit *(12 x 8/pk cartridges, 96 total with 1cc Cartridge Stand)*	186010126
Sep-Pak SEC Desalting Cartridges,1cc 5K MWCO *(12 x 8/pk cartridges, 96 total)*	186010127
1cc Cartridge Stand	186010128

The mechanism for separation is based on Size-Exclusion Chromatography (SEC), also known as Gel Filtration, where analytes are separated based on their size (in solution). Larger molecular weight analytes pass through the chromatographic bed and elute first and smaller molecular weight analytes spend more time diffusing in and out of the particle's pores eluting at later times.

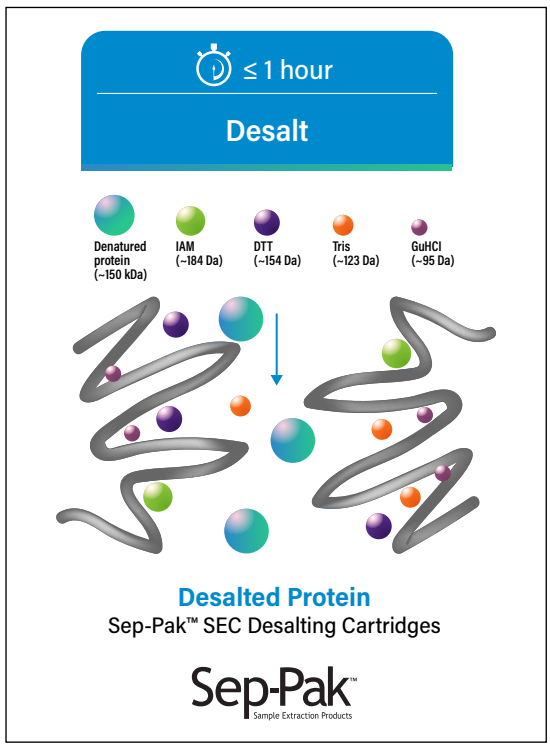

Figure 1. Schematic of Desalting by SEC

II. STORAGE AND STABILITY

The Sep-Pak SEC Desalting Cartridge should be stored at room temperature (≤ 25°C) and has a shelf-life of 11 months at room temperature (≤ 25°C).

III. USING SEP-PAK SEC DESALTING CARTRIDGES

Below are recommendations for preparing reagents (not provided) for use with the Waters PeptideWorks Tryptic Protein Digestion Kits. However, stocks can be prepared in accordance with your own protocols and preferences.

a. Exchange Buffer Selection and Preparation

Below are some examples of common exchange buffers used if performing protein digestion. Please note that you may use any suitable exchange buffer that is compatible with your protein.

1. 100 mM Tris-HCl with 10 mM $CaCl_2$, pH 7.5 (recommended).

   Prepare buffer according to the PeptideWorks Care & Use Manual (insert link when available) and proceed to trypsin digestion immediately after desalt.

2. 100 mM Tris-HCl, pH 7.5 followed by addition of $CaCl_2$ to a final concentration of 10 mM.

   Prepare buffer by diluting 1 M Tris-HCl (Sigma part number T2319 or equivalent) 10-fold with Milli-Q (18.2 MΩ) water. After desalt, spike the sample with 1 M CaCl2 Salt Concentrate (included in the PeptideWorks kits) to a final concentration of 10 mM $CaCl_2$ and proceed to digestion.

3. Waters Tris $CaCl_2$ Buffer Salts, pH 7.5 (p/n 186010110 1/pk or 186010111 4/pk).

   Prepare buffer by reconstituting according to the RapiZyme Trypsin Care & Use Manual and proceed to digestion immediately after desalt.

4. 100 mM Tris-HCl, pH 7.5 followed by dilution with reconstituted Waters Tris $CaCl_2$ Buffer Salts, pH 7.5.

   Prepare buffer by diluting 1 M Tris-HCl (Sigma part number T2319 or equivalent) 10-fold with Milli-Q (18.2 MΩ) water. After desalt, dilute the sample in a 1:1 ratio with reconstituted Tris $CaCl_2$ Buffer Salts, pH 7.5 (p/n 1860101101/pk or 186010111 4/pk) to a final concentration of ~5 mM $CaCl_2$ and proceed to digestion.

Reagent Stability Tip: Refrigerate this buffer in between uses and avoid leaving out at room temperature longer than 8 hours. It is common practice to store the refrigerated, diluted buffer for up to 6 months.

b. Extra Consideration: Desalting for GuHCl Removal

Desalting is highly recommended prior to trypsin digestion to remove high concentrations of GuHCl, which inhibits trypsin. Protocols that avoid desalting are possible, but suitability must be carefully studied for each protein.

Table 2. Checklist of Recommended Materials Required for Desalting and Buffer Exchange (Not Provided)

Material Description	Recommended Suppliers
Waste plate/tray	Waters p/n 600001282 or equivalent
QuanRecovery 96-well 700 uL plate	Waters p/n 186009184 or equivalent*
A collection vessel [UV-Vis compatible] with a flat-bottomed 96-well plate	Grenier p/n 655101 or equivalent

**Note: It is highly suggested to use a low-bind plate (like QuanRecovery) if sample is a peptide to prevent non-specific binding/adsorption losses that can occur with glass or standard polypropylene.*

c. Desalting and Buffer Exchange Protocol

1. Remove caps from Sep-Pak SEC Desalt Cartridge and let drain into waste plate.

Tip: Remove the top cap first, followed by the bottom.

2. Place cartridges in the cartridge stand above a waste plate.
3. Condition cartridges with 400 µL desalting/digestion buffer and let drain. Repeat twice more.
4. Add 100 µL of denatured, reduced, and alkylated protein and let drain. Move to the next step quickly.

Tip: Sample should not sit on the cartridge for longer than ten minutes before elution.

5. Add 100 µL of digestion buffer and let drain.
6. Replace waste reservoir with collection plate (96-well 700 µL collection plate).
7. Elute with 250 µL of digestion buffer.

Elution Tip: If higher protein recovery is desired (≥ 80%), it is suggested to elute with 300 µL of digestion buffer. The 250 µL volume was selected for optimal removal of GuHCl prior to an efficient tryptic digestion protocol.

8. Measure protein concentration and normalize to 0.1 mg/mL using digestion buffer.[1]

[1] *It is advised to check the desalted protein concentration to ensure accurate enzyme:protein ratio across samples if subsequent steps are digestion.*

Waters™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com