Agilent Peptide Cleanup C18 Spin Tubes

Instructions

Kit Contents

Fifty (50) Peptide Cleanup Spin Tubes, each containing 8 mg of C18 resin.

Each C18 spin tube can process a digest from ≥ 20 ng* of protein or ≤ 30 µg of total peptide in sample volumes from 10 to 150 µl. Recommended for cleanup of peptides from in-solution digests.

NOTE

*Sensitivity limits depend upon downstream analysis methods and equipment. Significant differences in detection limits exist among mass spectrometry (MS) systems.

Storage

Upon receipt, store at room temperature.

Introduction

Peptide samples can be purified and concentrated for a variety of applications using Agilent Peptide Cleanup C18 Spin Tubes. Each C18 spin tube contains a porous C18 reversed-phase resin with excellent binding and recovery characteristics at a wide range of peptide concentrations. The spin tube format allows simultaneous processing of multiple samples without the need for laborious repeated pipetting.

Matrix-assisted laser desorption ionization (MALDI-) and electrospray ionization (ESI-) mass spectrometry (MS) are vital tools for the study of biological compounds because of the high sensitivity and mass accuracy. MS methods are commonly used for examination of post-translational modifications and identification of proteins by peptide mapping. However, many of the buffers and compounds common to biological samples interfere with both MALDI-MS and ESI-MS. Peptide Cleanup C18 Spin Tubes remove interfering contaminants and release peptides in MS-compatible solutions, resulting in increased sensitivity and high-quality spectra. Although Peptide Cleanup C18 Spin Tubes are designed primarily for MS applications, they may be used for other applications such as peptide concentration and cleanup for peptide sequencing.

Procedure Summary

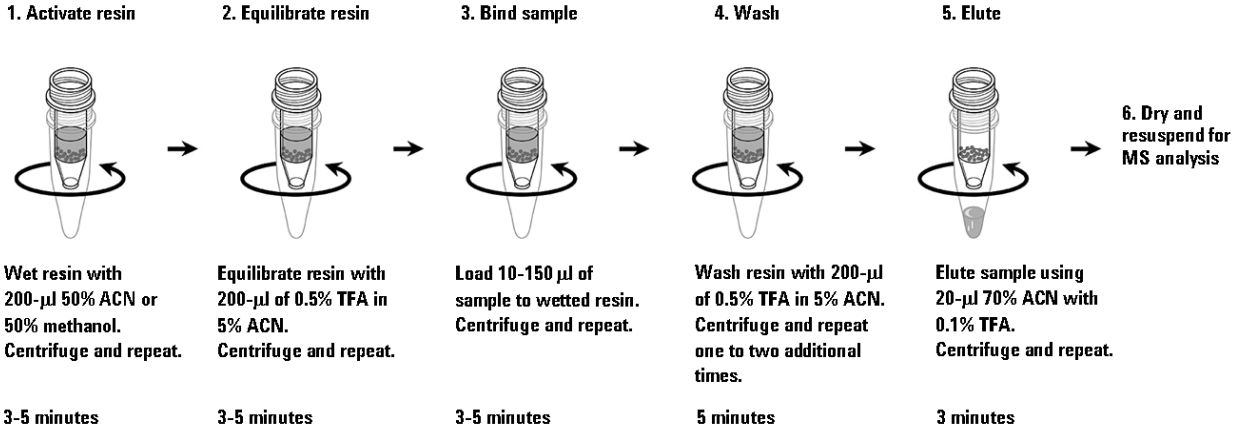

Figure 1 Peptide Cleanup C18 Spin Tube Procedure

Important Product Information

Please note the following:

- Peptide Cleanup C18 Spin Tubes are recommended for processing peptides derived from ≥ 20 ng (300 fmol) of protein or from samples containing from 0.5 ng to 30 µg of a singular peptide. Sample load requirements are largely dependent upon downstream analysis systems. Note: Significant differences in detection limits exist between MS systems.
- For binding to C18 reversed-phase resins, a sample must be free of excess organic solvents such as acetonitrile (ACN) or methanol. If organic solvents are present, dry the sample in a vacuum evaporator. Resuspend sample in 20–50 µl 0.5% trifluoroacetic acid (TFA) in 5% ACN before processing with Peptide Cleanup C18 Spin Tubes.
- For optimal results, proceed with the entire procedure in a timely manner and avoid excessive resin drying between steps.
- Plastics used during handling of peptide samples can introduce contaminants that interfere with MS analysis and result in sample loss from nonspecific adsorption. Use high-quality receiver tubes. If necessary, receiver tubes used for the final collection may be rinsed with 70% ACN/0.1% TFA before use. Minimizing sample transfers and freeze-thaws before analysis will help minimize plastic contamination and sample loss.

Additional Materials Required

The following is a list of required materials.

- Bench-top microcentrifuge capable of 3,000 × *g*
- Ultrapure water
- ACN
- TFA
- 1.5-ml microcentrifuge tubes
- Methanol (optional)

Material Preparation

Prepare the solution.

- Activation Solution: 50% ACN; 400 µl per sample

NOTE Methanol can be substituted for ACN.

- Equilibration/Wash Solution: 0.5% TFA in 5% ACN; 400 µl per sample for Equilibration plus 400–800 µl per sample for Washing (wash volume will be dependent upon amount and type of contaminants present in sample)
- Sample Buffer: 2% TFA in 20% ACN; 1 µl for every 3 µl of sample for samples that are in 100% water
- Elution Buffer: 70% ACN with 0.1% TFA; 40 µl per sample

NOTE The elution buffer used can be tailored to the downstream application. Acceptable buffers include 50%–70% ACN or 50%–70% methanol with or without 0.1% TFA. For ESI-MS analysis, replace TFA with 0.1% formic acid for best results.

Protocol for Sample Clean-up

Sample Preparation

Each Peptide Cleanup C18 Spin Tube can process 10–150 µl of sample. For 100% aqueous samples, mix three parts sample to one part Sample Buffer, so the final sample will contain 0.5% TFA in 5% ACN. For samples in other solutions, adjust content until the final sample solvent is approximately 0.5% TFA in 5% ACN or 5% methanol.

C18 Spin Tube Preparation

1. Tap C18 spin tube to settle resin. Remove top and bottom cap. Place C18 spin tube into a receiver tube.
2. Add 200 µl of Activation Solution to rinse walls of the C18 spin tube and to wet resin.
3. Centrifuge at 1,500 × *g* for 1 minute. Discard flow-through.
4. Repeat Steps 2 and 3.
5. Add 200 µl Equilibration/Wash Solution. Centrifuge at 1,500 × *g* for 1 minute. Discard flow-through.
6. Repeat Step 5.

Sample Binding

1 Load sample on top of resin bed.
2 Place C18 spin tube into receiver tube. Centrifuge at 1,500 × *g* for 1 minute.
3 To ensure complete binding, recover flow-through and repeat Steps 1 and 2.

NOTE Flow-through may be retained to confirm sample binding.

Wash

1 Place C18 spin tube into a receiver tube. Add 200 µl Equilibration/Wash Solution to C18 spin tube and centrifuge at 1,500 × *g* for 1 minute. Discard flow-through.
2 Repeat Step 1.

NOTE If sample contains high levels of contaminants (that is, 2 M urea or ≥ 100 mM ammonium bicarbonate), repeat the wash step one to two additional times.

Elution

1 Place C18 spin tube in a new receiver tube. Add 20 µl of Elution Buffer to top of the resin bed. Centrifuge at 1,500 × *g* for 1 minute.
2 Repeat Step 1 with same receiver tube.
3 Gently dry sample in a vacuum evaporator. For MALDI-MS analysis, carefully suspend sample in 1–2 µl of matrix solution prepared just before use. For LC-ESI applications, suspend sample in 0.1% formic acid or the appropriate buffer.

Troubleshooting

If experiencing a problem, refer to Table 1 below for possible causes and suggested solutions.

Table 1 Troubleshooting

Problem	Cause	Solution
Poor or incomplete sample binding	High pH, lack of ion-pairing agents	Ensure TFA was added to sample.
	Sample contains organic solvent	Dry sample and resuspend in 20 µl 0.1%–0.5% TFA.
	Sample not sufficiently hydrophobic to bind C18 resin	None
	Resin became dry before sample addition	Ensure resin does not dry during activation and equilibration of the resin; keep resin in Equilibration/Wash Solution until sample addition.
Poor or incomplete sample recovery	Highly hydrophobic sample	Use 70% ACN/0.1% TFA for elution.
	Sample loss due to nonspecific binding	Nonspecific binding of peptides to plastics can cause significant sample loss at very low peptide concentrations.
		Minimize contact with plastics and storage at low peptide quantities (that is, ≤ 300 fmol).
		Peptide Cleanup C18 Spin Tubes are not recommended for routine use at total peptide quantities of ≤ 300 fmol as special handling may be required.
	Detection limits of application	Ensure sample is within the detection limit of the specific downstream application. Note: Limits vary considerably based on application and instrumentation.

Related Agilent Products

See the following list for related Agilent products.

Product Number	Description
5188-2749	**Protein In-Gel Tryptic Digestion Kit,** sufficient reagents for up to 150 digests
5188-2747	**Lys Tag 4H Kit for MALDI-MS**, mass tagging reagent that improves sensitivity and mass spectrum quality for Lysine-containing peptides in MALDI-MS analysis
5188-2739	**Cleanup C18 Pipette Tips pk/96**, for purifying and concentrating peptides from in-gel digests
5188-2748	**Mass Tagging Accessories Kit**, kit containing products used in conjunction with mass tagging reagents for preparing samples for MS analysis (includes Cleanup C18 Pipette Tips, Tryptic Digestion Kit, MALDI matrix, and a peptide standard)
G2037A	**Alpha-Cyano-4-Hydroxycinnamic Acid**, MALDI Matrix 3 × 3 ml
5185-5984	**Multiple Affinity Removal Column, 4.6 × 50 mm,** depletes six high-abundance proteins from human serum samples, 15–20 µl serum capacity per injection
5185-5985	**Multiple Affinity Removal Column, 4.6 × 100 mm,** 30–40 µl serum capacity per injection
5185-2986	**Multiple Affinity Removal System Reagent Kit,** starter reagent kit containing buffers, spin filters, and spin concentrators for use with Multiple Affinity Removal Columns.

NOTE For Material Safety Data Sheets (MSDSs) and Certificates of Analysis, visit www.agilent.com/chem/msds.

Notices

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