INSTRUCTIONS

Pierce® Graphite Spin Columns

88302 2223.2

Number	Description
88302	**Pierce Graphite Spin Columns**, 30 columns, each column contains 10mg of graphite to purify up to 100µg of peptide Supplied: 0.5mL of slurry in 20% ethanol **Storage:** Upon receipt store at room temperature. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Graphite Spin Columns enable fast and efficient capture, concentration, desalting and elution of hydrophilic peptides. The procedure is simple and requires less than 20 minutes to process protein digests, strong cation exchange fractions, and enriched phosphopeptides for mass spectrometric (MS) analyses. Hydrophilic peptides, such as phosphopeptides, are difficult to purify by C18 columns. Pierce Graphite Spin Columns improve phosphopeptide analysis by efficiently binding hydrophilic peptides and removing hydrophobic peptides, urea, salts and other contaminants that interfere with MS analysis.

The graphite columns are ideal for matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) techniques. These MS methods are commonly used for examining post-translational modifications and identifying protein by peptide mapping; however, many buffers and compounds common to biological samples interfere with both MALDI and ESI. The Pierce Graphite Spin Columns increase sensitivity and improve MS spectra. The columns are also effective for other applications such as peptide concentration and clean-up for sequencing.

Important Product Information

- For optimal results, proceed with the entire procedure in a timely manner and avoid excessive graphite 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 microcentrifuge tubes. If necessary, tubes used for final collection may be rinsed with 70% acetonitrile/0.1% trifluoroacetic acid (TFA). Also, minimize sample transfers and freeze-thaw cycles before analysis.
- The Pierce Graphite Spin Columns (10mg) can bind up to 100µg of hydrophilic peptides. Use a sample loading volume of ≤ 500µL. Make sure samples are free of detergents before using the columns.

Procedure for Graphite Spin Columns

A. Additional Materials Required

- Sample: Dilute sample 1:1 in 2.5% TFA. Ensure the sample pH is 2.0-2.5 for proper binding.
- 100% Acetonitrile (200µL per sample)
- 1% TFA (400µL per sample)
- 0.1% TFA (20µL per sample)
- 1M NH_4OH (200µL per sample)
- 0.1% Formic acid in 50% acetonitrile (400µL per sample)
- 1.5mL Microcentrifuge tubes

B. Column Preparation

1. Remove top and bottom cap, place column into a 1.5mL collection tube and centrifuge at 2000 × *g* for 1 minute to remove storage buffer.
2. Add 100µL of 1M NH_4OH and centrifuge at 2000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
3. Activate graphite by adding 100µL of acetonitrile. Centrifuge at 2000 × *g* for 1 minute and discard flow-through.
4. Add 100µL of 1% TFA and centrifuge at 2000 × *g* for 1 minute. Discard flow-through. Repeat this step once.

C. Sample Binding and Elution

1. Place column into a new collection tube and apply sample on top of the resin bed. Allow binding for 10 minutes with periodic vortex mixing.
2. Centrifuge at 1000 × *g* for 3 minutes. Discard the flow-through.
3. Place column into a new collection tube. Wash column by adding 200µL of 1.0% TFA and centrifuging at 2000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
4. Place column into new collection tube. Add 100µL of 0.1% formic acid in 50% acetonitrile to elute sample. Centrifuge at 2000 × *g* for 1 minute. Repeat this step three more times using the same collection tube for a total elution volume of 400µL.

   Optional: To reduce final volume, elute with 100µL of 0.1% formic acid in 50% acetonitrile. Re-apply eluate three more times for a total elution volume of 100µL.
5. Gently dry sample in a vacuum evaporator. For MALDI-MS analysis, carefully suspend sample in 1-2µL of matrix solution prepared just before use. For LC-ESI applications, suspend sample in 0.1% TFA or the appropriate buffer.

Troubleshooting

Problem	Possible Cause	Solution
Poor or incomplete sample binding	High pH, lack of ion-pairing agents	Ensure that TFA was added to sample before binding
	Lack of mixing during binding	Ensure that graphite is suspended in sample during binding
Poor or incomplete sample recovery	Peptides binding to plastics can cause significant loss at low peptide concentrations	Minimize contact with plastics, excessive drying and storage at low concentration
	Detection limits of the specific application	Ensure sample is within the detection limit of the specific application – limits vary considerably based on application and instrumentation
Graphite material is present in the flow-through or washes	A detergent or detergent-like compound was in the sample	Remove detergent from the sample with detergent-removal columns (Product No. 87777) or remove detergent from the protein sample by acetone precipitation or detergent-removal columns before digestion

Related Thermo Scientific Products

20062 **Acetonitrile**, 50mL

53102 **Trifluoroacetic Acid, HPLC grade**, 10 × 1mL

90003 **Pierce Phosphoprotein Enrichment Kit**

88300 **Pierce Fe-NTA Phosphopeptide Enrichment Kit**

88301 **Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit**

88811 **Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit**

87777 **Pierce Detergent Removal Spin Column, 0.5mL,** 25 columns

87784 **Pierce C18 Tips,** 100µL, 96 tips

89870 **Pierce C18 Spin Columns**, 25 columns

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.