INSTRUCTIONS

Pierce® Fe-NTA Phosphopeptide Enrichment Kit

88300

2170.0

Number	Description
88300	**Pierce Fe-NTA Phosphopeptide Enrichment Kit,** 30 columns
	Kit Contents:
	Spin Columns, 30 columns, each column contains 200 µl of resin slurry
	Binding Buffer, 6 ml
	Wash Buffer (2X), 12 ml
	Elution Buffer, 6 ml
	Storage: Upon receipt store at 4°C. Product shipped with ice pack.

Introduction

The Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit enables fast and efficient enrichment of phosphorylated peptides. The procedure is simple and requires less than 30 minutes to process protein digests and strong-cation exchange fractions for analyses by mass spectrometry (MS). Each spin column included in the kit contains phosphopeptide-specific resin that offers excellent binding and recovery properties for enriching up to 150 µg of phosphopeptides.

Mass spectrometry is a key tool for identifying and quantitating phosphorylation changes. Matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometry are vital tools for studying biological compounds because of the high sensitivity and mass accuracy. Although these tools are powerful, mass spectrometric analysis of phosphoproteins and phosphopeptides are often limited by factors such as inadequate digestion efficiency, low stoichiometry, hydrophilicity and poor ionization and fragmentation. Enrichment is essential to successful MS analysis of phosphopeptides. These new high-capacity Fe-NTA spin columns included in this kit complement our lysis, reduction, alkylation and digestion reagents and C18 and graphite spin columns to provide a complete workflow for phosphopeptide enrichment.

Important Product Information

- For optimal results, proceed with 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, low-binding receiver tubes. If necessary, rinse receiver tubes used for final collection with 70% acetonitrile/0.1% trifluoroacetic acid. Minimizing sample transfers and freeze-thaw cycles before analysis will help reduce plastic contamination and sample loss.
- Avoid using solutions that contain > 5 mM EDTA or other reducing agents.
- For best results, clean-up of elution fractions containing phosphopeptides is recommended prior to MS analysis. Phosphopeptides and other hydrophilic peptides bind efficiently to porous graphite resin, providing a better method for rapid desalting and concentration in comparison to C18 media. The use of Pierce Graphite Spin Columns (Product No. 88302) is strongly recommended for processing samples after enrichment.

Additional Materials Required

- Mass spectroscopy-grade acetonitrile
- Trifluoroacetic acid (TFA)
- Ultrapure water

Material Preparation (per spin column)

Wash Buffer A	Wash Buffer 2X Stock	150 µl
	Ultrapure water	150 µl
	Total	300 µl
Wash Buffer B	Wash Buffer 2X Stock	150 µl
	Acetonitrile	30 µl
	Ultrapure water	120 µl
	Total	300 µl

Procedure for Phosphopeptide Enrichment

Note: Specific samples might require optimization. Please read entire instructions before using this product.

A. Sample Preparation

1. Quick dry sample in a rotary vacuum concentrator (e.g., Thermo Scientific SpeedVac Vacuum Concentrator). Resuspend dried sample in 200 µl of Binding Buffer
2. Add sample to a Fe-NTA spin column and incubate for 20 minutes at room temperature with end-over-end rotation. Remove bottom tab of the column. Place column in a microcentrifuge tube.
3. Centrifuge column at 1,000 × *g* for 1 minute. Transfer column to new collection tube. If desired, retain flow-through for analysis.

B. Wash

1. Add 100 µl of Wash Buffer A to the spin column and gently mix contents.
2. Centrifuge column at 1,000 × *g* for 1 minute. Transfer column to new collection tube. If desired, retain flow-through for analysis.
3. Repeat steps B1 and B2 once.
4. Add 100 µl of Wash Buffer B to the spin column and gently mix contents.
5. Centrifuge tube at 1,000 × *g* for 1 minute. Transfer column to new collection tube. If desired, retain flow-through for analysis.
6. Repeat steps B4 and B5 once.
7. To help equilibrate resin for elution, add 100 µl of ultrapure water and gently mix contents. Centrifuge column at 1,000 × *g* for 1 minute. Transfer column to a new collection tube.

C. Phosphopeptide Elution

1. Add 50 µl of Elution Buffer to the resin. Incubate for 3-5 minutes at room temperature.
2. Centrifuge column at 1,000 × *g* for 1 minute. Retain phosphopeptide eluate for analysis.
3. Repeat steps C1 and C2 two additional times. If desired, pool the elution fractions.
4. Acidify elution by adding 200 µl of 2.5% TFA.

   Note: For best results, process the elution fraction with the Pierce Graphite Spin Columns (Product No. 88302) before MS analysis.
5. Gently dry samples in vacuum evaporator. For LC-ESI applications suspend sample in 20-50 µl of 5% Formic acid or appropriate buffer.

Troubleshooting

Problem	Possible Cause	Solution
Nonspecific peptide binding	pH of sample or Wash Buffers is > 3.5	Check pH and adjust to < 3.5 by increasing acetic acid concentration
	Wash volume is insufficient for peptide concentration	Increase the number or volume of washes
	Nonspecific peptide is highly acidic	Increase acetic acid concentration or add 100 mM sodium chloride to Wash Buffer **Note:** Sample will require desalting before MS analysis.
	Nonspecific peptide is highly hydrophobic	Increase amount of acetonitrile in Wash Buffer to 30%
No phosphopeptide recovered	Phosphopeptide concentration is too low	Increase sample amount added to the column or reduce Elution Buffer volume
	MS analysis results are poor	Some phosphopeptides can be difficult to detect by MS
Phosphopeptides did not bind	Sample is overly buffered and pH remains > 5.5	Modify sample preparation method if possible
	High levels of interfering agents such as EDTA, free metals or ammonium bicarbonate are in the sample	Modify sample preparation method if possible

Related Thermo Scientific Products

88302 **Pierce Graphite Spin Columns,** 30 columns

53102 **Trifluoroacetic Acid, HPLC Grade,** 10 × 1 ml

20062 **Acetonitrile,** 50 ml

90003 **Pierce Phosphoprotein Enrichment Kit**

87700 **Grb2 SH2 Domain Phosphotyrosine Capture Kit**

87701 **Src SH2 Domain Phosphotyrosine Capture Kit**

87715 **Syk SH2 Domain Phosphotyrosine Capture Kit**

87716 **PI3K SH2 Domain Phosphotyrosine Capture Kit**

78420 **Halt Phosphatase Inhibitor Cocktail,** 1 ml

78426 **Halt Phosphatase Inhibitor Cocktail,** 5 × 1 ml

78428 **Halt Phosphatase Inhibitor Single-Use Cocktail,** 24 × 100 µl

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