Simple and efficient enrichment of phosphopeptides

Introducing a new Fe-NTA format optimized for capture and recovery

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The new Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit enables fast and efficient enrichment of phosphorylated peptides. These spin columns are easy to use and require less than 1 hour to process protein digests or strong cation-exchange peptide fractions for analysis by mass spectrometry (MS).

Highlights:

- Convenient spin format for parallel processing of multiple samples
- High-binding capacity resin for enriching up to 150µg of phosphopeptides per column
- Excellent enrichment and recovery of phosphopeptides

Introduction

Protein phosphorylation is essential to biological functions, including cell signaling, growth, differentiation, division and programmed cell death. Over 500 protein kinases catalyze phosphorylation of specific targets, primarily on serine, threonine, and tyrosine residues.

Mass spectrometry is increasingly being used to identify and quantify phosphorylation changes; however, phosphoprotein and phosphopeptide analysis by MS is limited by many factors, including digestion efficiency, low stoiochiometry, low abundance, hydrophilicity, poor ionization and poor fragmentation. As a result, phosphopeptide enrichment is essential to successful MS analysis. The new Pierce* Fe-NTA Phosphopeptide Enrichment Kit is compatible with our lysis, reduction, alkylation and digestion reagents and with Thermo Scientific Pierce Graphite Spin Columns to provide a complete workflow for phosphopeptide enrichment.

Methods

To assess phosphopeptide enrichment from lysates, cultured U2-OS cells arrested with nocodazole (100ng/ml, 25 hours) were lysed with 6M urea in 50mM Tris, pH 8.0 containing Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail (Product # 78440). Protein concentration was determined with Thermo Scientific Pierce 660nm Protein Assay (Product # 22660). Proteins were reduced with Thermo Scientific Bond-Breaker TCEP Solution, Neutral pH (Product # 77720), alkylated with single-use iodoacetamide (Product # 90034), digested overnight with MS-grade trypsin (Product # 90055), and desalted with Thermo Scientific HyperSep-C18 Cartridges (Product # 60108-305). An equivalent of 200µg of peptides were dried and dissolved in 5% acetic acid or Sigma Phos-Select* Buffer. Phosphopeptides were enriched with Pierce Fe-NTA Phosphopeptide Enrichment Kit or Sigma Phos-Select Reagents and then desalted and concentrated with Pierce Graphite Spin Columns (Product # 88302) according to manufacturers' instructions.

Enriched phosphopeptide samples were analyzed by LC-MS/MS. A NanoLC*-2D HPLC (Eksigent) with a ProteoPep II* C18 Column (75µm ID x 20cm, New Objective) was used to separate peptides using a 4-40% gradient of solvents (A: water, 0.1% formic acid; B: acetonitrile, 0.1% formic acid) at 250nl per minute for 60 minutes. Peptides were identified with a Thermo Scientific LTQ Orbitrap XL ETD Mass Spectrometer using a top four experiment consisting of high-resolution MS followed by acquisition of four MS/MS spectra using the CID mode of fragmentation. LC-MS/MS data were interpreted with Mascot 2.2 (Matrix Science) and Scaffold 2.6 (Proteome Software).

Results

To achieve robust MS results, enrichment of phosphopeptide samples is essential because of low stoichiometry and abundance and poor ionization relative to nonphosphorylated peptides. We have developed an efficient means to enrich phosphopeptides from complex samples. The new Thermo Scientific Pierce Fe-NTA Spin Columns effectively capture, enrich and recover phosphopeptides. These columns enrich a higher percentage of phosphopeptides than other resins and with an overall higher number of total and unique phosphopeptides (Figure 1 and Table 1).

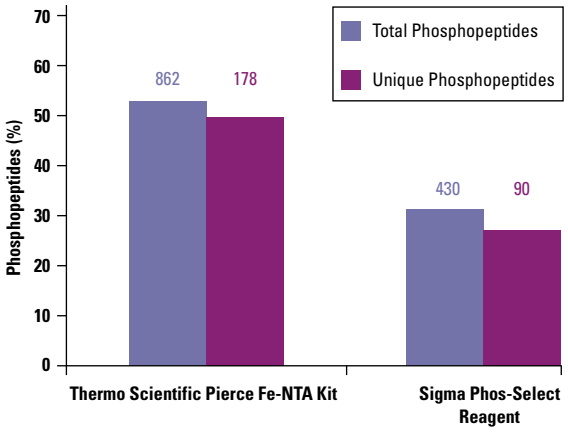

Figure 1. Our kit enriched a greater percentage of total and unique phosphopeptides from U2-OS cell lysate. Numbers refer to the total and unique number of phosphopeptides identified in each condition. A summary of results is listed in Table 1.

Table 1. Average phosphopeptide enrichment results from duplicate experiments.§

	Thermo Scientific Pierce Fe-NTA	Sigma Phos-Select Reagent
Total phosphopeptides	862	430
Total peptides	1,753	1,665
Total unique peptides	393	395
Total unique phosphopeptides	178	90
Total phosphopeptides (%)	53	31
Unique phosphopeptides (%)	50	27.5

§ Peptide summary results were exported from Scaffold and analyzed and summarized with Microsoft Excel and Access*.*

Multiple phosphorylated amino acids within a peptide contribute to the complexity of phosphopeptide analysis. Pierce Fe-NTA Spin Columns enrich peptides with three or more phosphorylated sites and significantly outperform other commercially available columns (Figure 2). Phosphopeptide enrichment greatly reduces sample complexity and enables effective identification and characterization of phosphorylated peptides by MS (Figure 3).

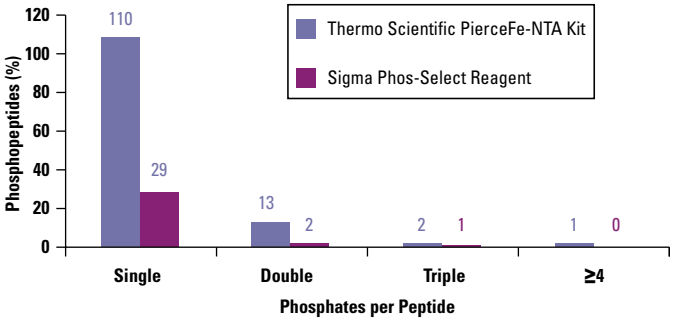

Figure 2. The Thermo Scientific Pierce Fe-NTA Phosphopeptide Enrichment Kit effectively captures phosphopeptides with multiple phosphates.

The Pierce Fe-NTA Phosphopeptide Enrichment Kit contains detailed instructions and all necessary components to load, wash and elute phosphopeptides within an hour. This kit is compatible with samples digested in solution or after in-gel digestion using the Thermo Scientific In-gel Tryptic Digestion Kit (Product # 89871).

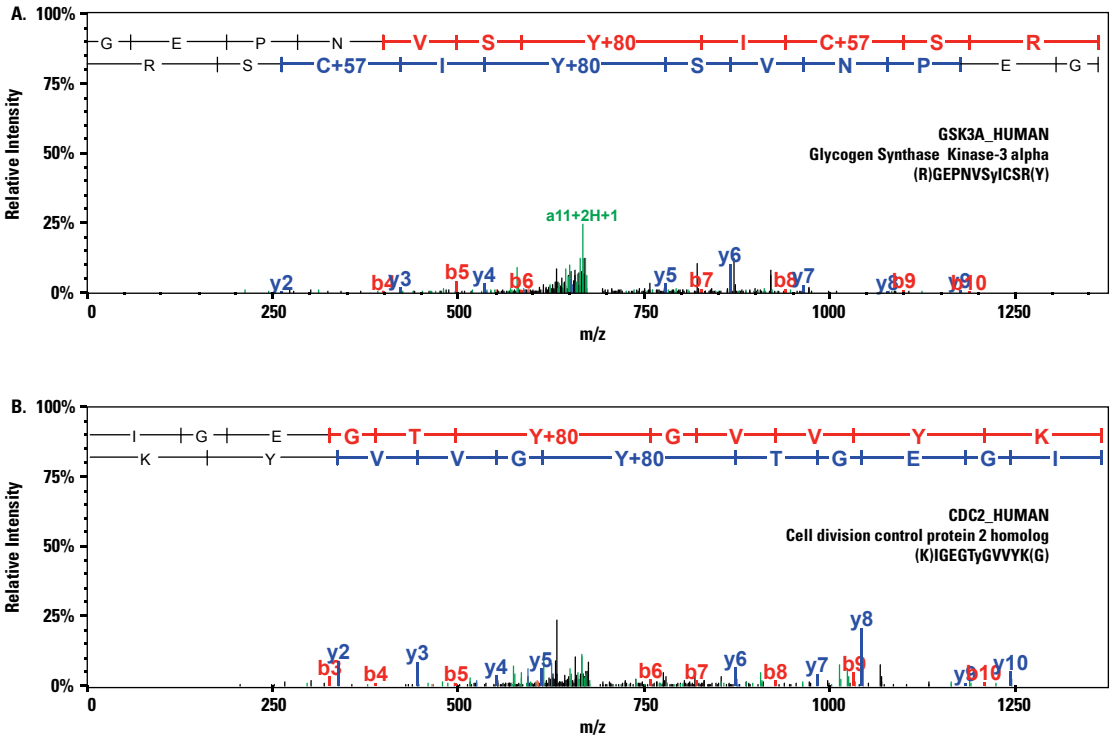

Figure 3. Example MS/MS spectra from enriched phosphopeptides. Lowercase letters indicate the position of tyrosine phosphorylation in enriched peptides. **Panel A:** Glycogen synthase-3 alpha. **Panel B:** cdc2/cyclin dependent kinase 1.

Ordering Information

Product #	Description	Pkg. Size
88300	**Pierce Fe-NTA Phosphopeptide Enrichment Kit** *Sufficient for 30 samples.*	Kit

Related Products

88302	**Pierce Graphite Spin Columns 0.5ml, 10mg/column**	30 columns

** Trademark, see trademark index on page 23.*