

POST-TRANSLATIONAL MODIFICATIONS

Phosphorylation

Reversible phosphorylation is one of the most important and well-studied post-translational modifications. Most commonly occurring on threonine, serine, and tyrosine residues, phosphorylation plays critical roles in the regulation of many cellular processes including: cell cycle, growth, apoptosis and differentiation. Thus, the identification and characterization of phosphorylation sites is crucial for the understanding of various signaling events. Mass spectrometry (MS) of phosphopeptides obtained from tryptic protein digests has become a powerful tool for characterization. However, there is a general need to significantly enrich samples for phosphopeptide content in order to compensate for low abundance, poor ionization, and suppression effects.

Immobilized metal affinity chromatography (IMAC) has been commonly used for purification of phosphorylated compounds. PHOS-Select™ Iron Affinity Gel and PHOS-Select™ HC Iron-coated 96-well Plate are prepared with a novel iron [Fe(III)] chelate matrix based on our proprietary (patent pending) NTA analog chelate ligand. This matrix provides high capacity affinity binding of molecules containing phosphate groups, making these products ideal for the enrichment of phosphopeptides from protein tryptic digests, or small organic phosphocompounds (adenosine 5'-monophosphate). They can also be used for direct transfer of phosphocompounds for analysis by HPLC or mass spectrometry.

PHOS-Select™ Iron Affinity Gel

An exceptionally efficient chemistry for phosphopeptide enrichment

Features & Benefits

- High capacity (at least 4 micromoles of phosphopeptide per ml of resin)
- Precharged with iron (III) as a stable chelate
- Provides single-step phosphocompound purification



Resin Elution Selectivity - MALDI-MS

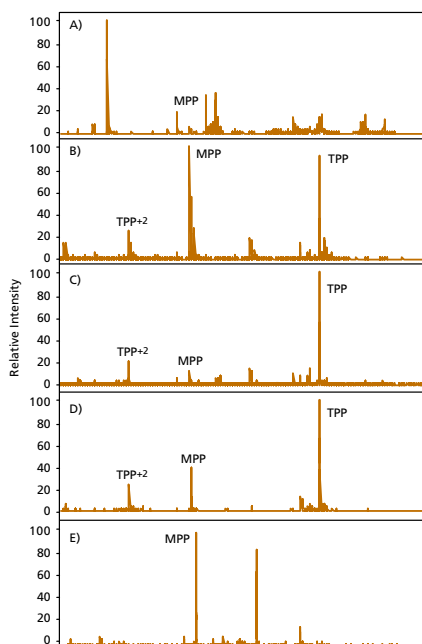


Figure 1. MALDI mass spectra of A) Crude β -casein tryptic digest (113 pmol/ μ L) prior to loading on the PHOS-Select Resin and B)-E) purified phosphopeptides after elution from the PHOS-Select Resin under various elution conditions. Eluents were as follows: B) 0.15 M NH_4OH , C) 0.4 M NH_4OH , neutralized with formic acid prior to analysis, D) 0.15 M NH_4OH , 25% ACN, and E) 5% formic acid, 25% ACN.

Resin Enrichment - HPLC

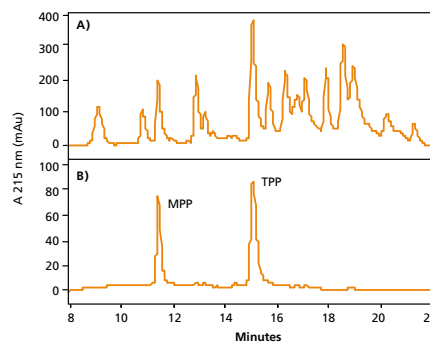
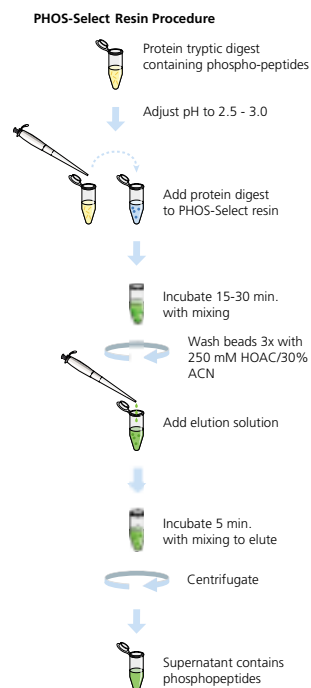


Figure 2. A) HPLC analysis of crude β -casein tryptic digest (113 pmol/ μ L, 5 μ L injection) prior to loading on the PHOS-Select Resin. B) HPLC analysis of purified phosphopeptides after elution from the PHOS-Select Resin using 500 μ L of 0.15 M NH_4OH , 25% ACN.

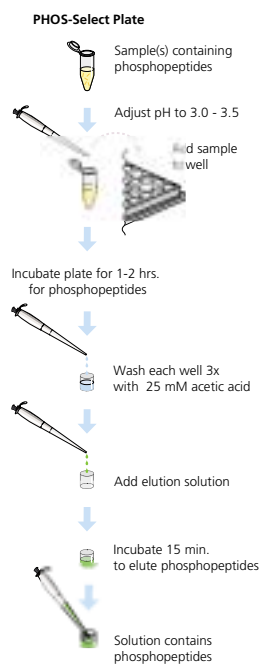
MPP - Monophosphopeptide, monoisotopic
 $M+H^+ = 2061.8212$

TPP - Tetraphosphopeptide, monoisotopic
 $M+H^+ = 3122.2584$



Product Code	Description	Size
P 9740	PHOS-Select Iron Affinity Gel	5 ml
P 9615	Phosphopeptide Positive Control Set	1 set

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PHOS-Select™ HC Iron-Coated 96-Well Plate

Features & Benefits

- Coated with a high density iron chelate in a large pore matrix
- High capacity (>300 picomoles of phosphopeptide per well)
- Maximum recommended working volume of 200 μ l per well

Plate Enrichment - HPLC

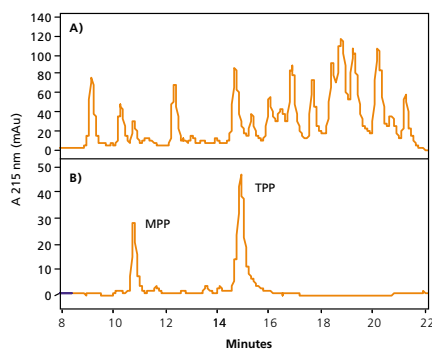


Figure 1. A) HPLC analysis of crude β -casein tryptic digest (4.5 pmol/ μ L, 50 μ L injection) prior to loading on the PHOS-Select Plate. B) HPLC analysis of purified phosphopeptides after elution from the PHOS-Select Plate using 200 μ L of 0.15 M NH_4OH .

Plate Elution Selectivity - MALDI-MS

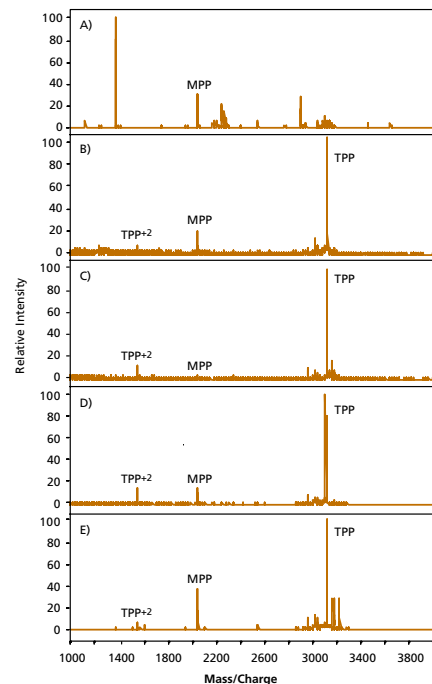


Figure 2. MALDI mass spectra of A) Crude β -casein tryptic digest (4.5 pmol/ μ L) prior to loading on the PHOS-Select Plate and B)-E) purified phosphopeptides after elution from the PHOS-Select Plate under various elution conditions.

Product Code	Description	Size
P.1617	PHOS-Select HC Iron-coated 96-well Plate	1 plate
P.9615	Phosphopeptide Positive Control Set	1 set

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Additional Products for Phosphoprotein Analysis

Antibodies for the Isolation of Phosphopeptides

Product Code	Name
A 1806	Monoclonal Anti-Phosphotyrosine Agarose, clone PT-66
A 8076	Monoclonal Anti-Phosphoserine Agarose, clone PSR-45
A 7951	Monoclonal Anti-Phosphothreonine Agarose, clone PTR-8

Antibodies for the Detection of Phosphopeptides

Product Code	Name
A 3588	Monoclonal Anti-Phosphotyrosine, clone PT-66, purified mouse Immunoglobulin
P 3430	Monoclonal Anti-Phosphoserine, clone PSR-45, mouse ascites fluid
P 3300	Monoclonal Anti-Phosphotyrosine, clone PT-66, mouse ascites fluid
A 4595	Monoclonal Anti-Phosphotyrosine, peroxidase conjugate, clone PY20
A 5964	Monoclonal Anti-Phosphotyrosine, peroxidase conjugate, clone PT-66
B 1531	Monoclonal Anti-Phosphotyrosine, Biotin conjugate, clone PT-66
B 5055	Monoclonal Anti-Phosphotyrosine, Biotin conjugate, clone PY20
F 0426	Monoclonal Anti-Phosphotyrosine, FITC conjugate, clone PY20
F 3145	Monoclonal Anti-Phosphotyrosine, FITC conjugate, clone PT-66
P 4110	Monoclonal Anti-Phosphotyrosine, clone PY20, purified mouse Immunoglobulin
T 6043	Monoclonal Anti-Phosphotyrosine, TRITC conjugate, clone PT-66
B 7911	Monoclonal Anti-Phosphoserine, Biotin conjugate
F 3662	Monoclonal Anti-Phosphoserine, FITC conjugate
P 3555	Monoclonal Anti-Phosphothreonine, clone PTR-8, mouse ascites fluid
B 7661	Monoclonal Anti-Phosphothreonine, Biotin conjugate
F 3787	Monoclonal Anti-Phosphothreonine, FITC conjugate

Reagents and Positive Controls

Product Code	Name	Description
A 7302	Ammonium molybdate	Used in Taussky Shorr assay of inorganic phosphate
B 2507	Barium hydroxide	Dephosphorylation of Phosphopeptides for MS (see Molloy and Andres, 2001)
C 6780	α -Casein	Positive control, phosphorylated at serines
C 6905	β -Casein	Positive control, phosphorylated at serines
A 5503	Ovalbumin	Positive control, phosphorylated at serines
P 1253	Phosvitin	Positive control, phosphorylated at serines
F 7002	Ferrous sulfate	Used in Tosky Shorr assay of inorganic phosphate
P 7751	Phenylphosphate	Elution of Phosphoproteins from antibody resins
P 9615	Phosphopeptide Positive Control Set	Standards for phosphopeptide purification and characterization
P 3717	Phosphoserine-BSA	Inhibitor of anti-phosphoserine for blotting and ELISA
P 3842	Phosphothreonine-BSA	Inhibitor of anti-phosphothreonine for blotting and ELISA
P 3967	Phosphotyrosine-BSA	Inhibitor of anti-phosphotyrosine for blotting and ELISA
M 9636	Malachite Green	Use in high-sensitivity inorganic phosphate analysis