

Phosphorylated Protein Research

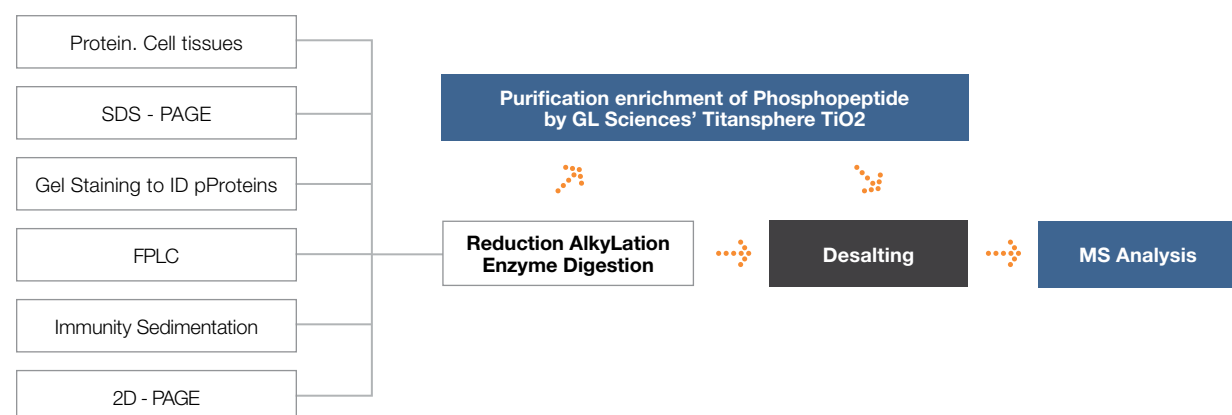
Protein phosphorylation is recognized as a fundamental process which regulates cell differentiation, growth, and migration. Analyzing protein phosphorylation is complicated by the low concentration of any given phosphoprotein and any one time, and the relatively low ionization efficiency of phosphopeptides in MS analysis. Therefore, enrichment of phosphopeptides and the relative reduction of non-phosphorylated peptides is critical to accurate analysis of protein digests by LC/MS.

GL Sciences' Titanium Dioxide (TiO₂ or Titania) products have emerged as the most effect means of phosphopeptide enrichment of protein digests prior to LC/MS analysis, replacing IMAC as the primary means of phosphopeptide sample pretreatment. Enrichment by titanium dioxide and IMAC, remain, however, complimentary techniques and are often used in combination to obtain optimal phosphopeptide analysis.

What Makes GL Sciences' Titanium Dioxide Products Unique and Superior?

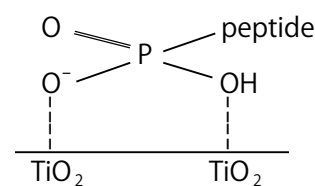
Titanium Dioxide exists in three crystalline forms, known as rutile, anatase, and brookite. Rutile and Anatase forms are the most common and most useful for phosphopeptide enrichment, and the ratio of rutile form to anatase form has significant implications for applicability to enrichment of phosphopeptides. GL Sciences' manufacturing technique for it's phosphopeptide enrichment products produces a highly spherical bead with the optimum ratio of crystal forms of TiO₂. The primary reasons the GL Sciences' Pho-TiO and MonoTip products show superior performance is a direct result of the unique formulation of our titanium dioxide beads.

Basics of Phosphopeptide Analyses by MS



Principal of Phosphopeptide Enrichment using GL Sciences' Phos-TiO Sample Enrichment Products

Adsorption Mechanism

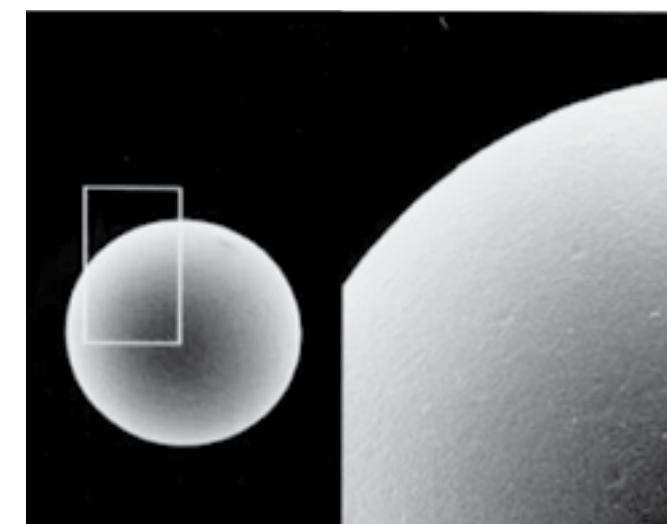


Phosphate groups are preferentially adsorbed to the surface of titanium dioxide under acidic conditions and are eluted under basic conditions. Non-phosphorylated acid peptides non-specifically bound to the TiO₂ can be reduced by adding acid modifiers to the loading and/or wash buffers.

Bulk Sorbent Materials for Purification & Enrichment of Phosphopeptides

Titansphere™ TiO Bulk Material

While GL Sciences' Phos-TiO spin columns and MonoTip TiO pipette tip based enrichment products are useful for most sample pretreatment applications, some investigators require bulk titanium dioxide media for specialized applications. Titansphere TiO bulk sorbent media is available in 5u and 10u particle sizes in quantities of 500mg.



Purification/Enrichment Protocol

Phos-TiO centrifugation spin columns require only 5 steps:

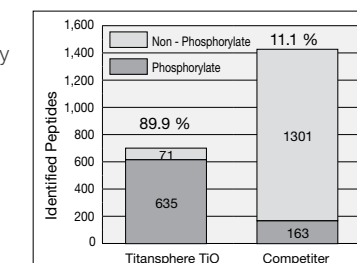


Application

Efficient purification from HeLa Cell Lysate

The data at right shows the superior performance of Titansphere TiO using the HeLa Cell Lysate consisting mainly of non-phosphorylated peptides. Titansphere TiO shows exceptional selectivity - almost 90% of the bound peptides were phosphopeptides, and excellent capacity for total phosphopeptide binding. A competitive TiO product is shown, binding mainly non-phosphorylated peptides and a much lower total number of discreet phosphopeptide species.

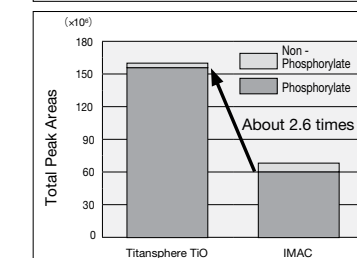
Sample: HeLa Cell Lysate, Sample volume: 50 µg, Titansphere TiO volume: 1 mg



Compare Titansphere TiO with IMAC

The graph at lower right shows how Titansphere TiO compares to an IMAC enrichment using Arabidopsis cell extract. Titansphere TiO provides substantially higher total capacity and a much higher number of discreet phosphopeptides isolated.

Sample: Arabidopsis Cell Extract, Sample volume: 100 µg, Titansphere TiO volume: 1 mg



Specifications

Description	Titansphere TiO
Particle Size	5 µm, 10 µm
Particle Shape	Spherical
Adsorption Spot	Titanium Dioxide Crystal
Pore Size	100 Å (10 µm)
pH Range	2 ~ 12
Gravity	1.74

Identified Numbers of Phosphopeptides

	Phosphorylate	Non - Phosphorylate
Titansphere TiO	846	198
IMAC	474	379

Titansphere™ TiO Part Number

Description	Volume	Cat.No.
Titansphere TiO 5 µm	500 mg	5020-75000
Titansphere TiO 10 µm	500 mg	5020-75010

PHOSPHORYLATION PURIFICATION & ENRICHMENT

Enrichment of Phosphopeptide Using Spin Columns

Titansphere™ Phos-TiO Kit

Titansphere Phos-TiO kits contain titansphere media in a tip-column designed for use with centrifugal solution flow. These spin columns offer the same TiO material provided in bulk form in convenient 200 μ L (3 mg TiO) and 10 μ L (1 mg TiO) sizes, and include waste and collection tubes as well as all required buffers.



Features

Easy to Operate

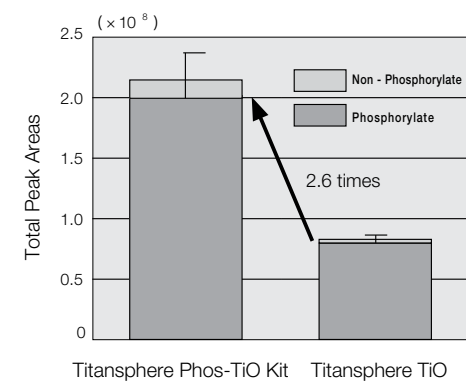
Only 5 steps (completed in about 40 minutes) are required for sample enrichment. Many individual samples can be processed simultaneously without cross contamination.

Phosphopeptide Loading Capacity

Description	Content	
Sample	Tyr (PO ₃ H ₂) - Angiotensin II	
Tip Column	3 mg/200 μ L	1 mg/10 μ L
Loading Volume	3.5 μ g	1.2 μ g

Performance

Optimal TiO beads are used for Titansphere Phos-TiO Kit.



The existing Titansphere TiO beads were improved for better adsorption capacity of phosphopeptides. Compared to the existing Titansphere beads, Phos-TiO Kit showed 2.6 times more peak area and 1.6 times more identified phosphopeptides.

Sample: HeLa Cell Lysate
Sample Volume: 50 μ g
Titansphere TiO beads: 1 mg

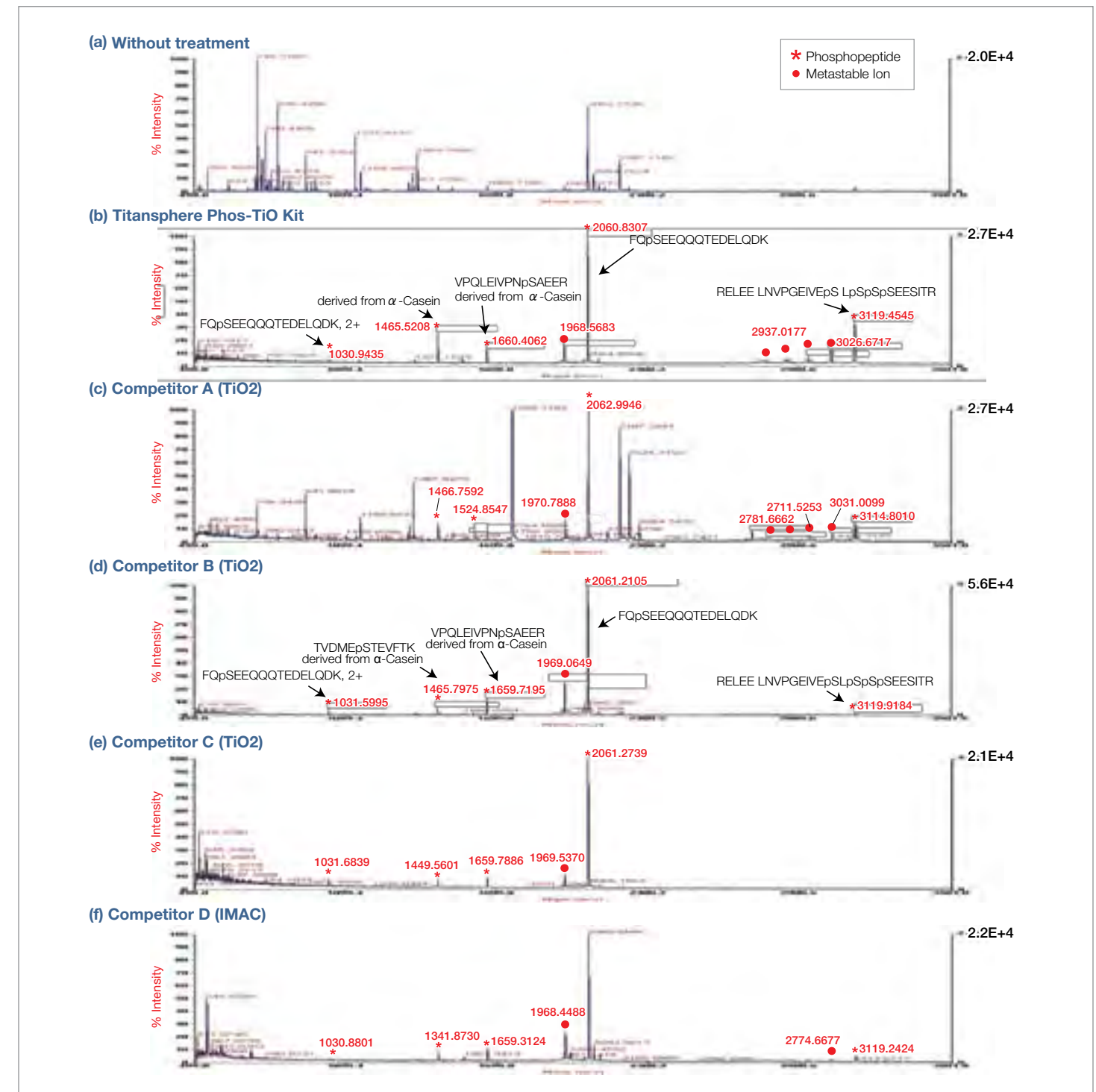
Identified Numbers of Phosphopeptides

	Phosphorylate	Non-Phosphorylate
Titansphere Phos-TiO Kit	996	185
Titansphere TiO	635	71

Titansphere Phos-TiO Kit was developed based on the cooperation from Dr. Yasushi Ishihama from Graduation School of Pharm Sci, Kyoto University.

Application

Fig. 1 - Phos-TiO Kits outperform 4 competitive TiO based products for phosphopeptide enrichment (MALDI-TOF/MS)



The data above show the purification efficiency of various TiO based products with a 2.5 μ g sample of B-casein digest using MALDI-TOF/MS. Compared to the untreated condition (a), phosphopeptides were selectively purified when using Titansphere Phos-TiO Kit. Compared to competitive products (c - e) Titansphere Phos-TiO Kit showed better selectivity. In general titanium dioxide is said that it has the worse adsorption efficiency of multi-phosphopeptides than IMAC. However, Titansphere Phos-TiO Kit showed higher selectivity, sensitivity and number of individual phosphopeptides isolated for 4 - phosphopeptides than IMAC (f)

Metastable Ion is a dephosphorylated peak.

PHOSPHORYLATION PURIFICATION & ENRICHMENT

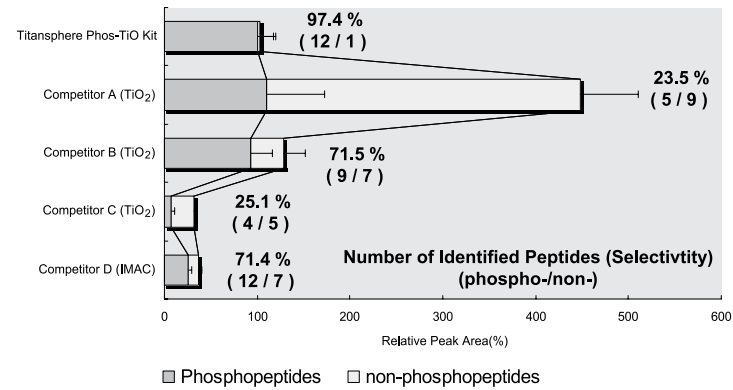
Application

Fig. 2 Comparison Between Titansphere Phos-TiO Kit and 4 Other Market Available Phosphopeptides Enrichment Methods (LC-MS)

Tryptic digest of a-casein, Futein and Phosvitn (each 2.5 µg) were used compare to observe the purification efficiency.

The peak area value of phosphopeptides purified by Titansphere Phos-TiO Kit is shown as 100% (n=3)

The % shown in the Fig. 2 is ratio of phosphopeptides peak area value in the detected peptides peak area value. Also (%) in the Fig. 2 shows the numbers of identified peptides (phosphopeptides/non-phosphopeptides)



Titansphere™ Phos-TiO for Large Volume Samples

GL Sciences now introduces larger versions of these spin columns as an extension of the Phos-TiO product line, including a 3 mL column containing 50 mg of our unique titanium dioxide (TiO₂), and another column containing 100 mg of our TiO₂.

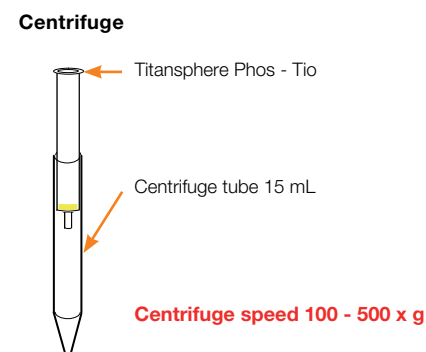
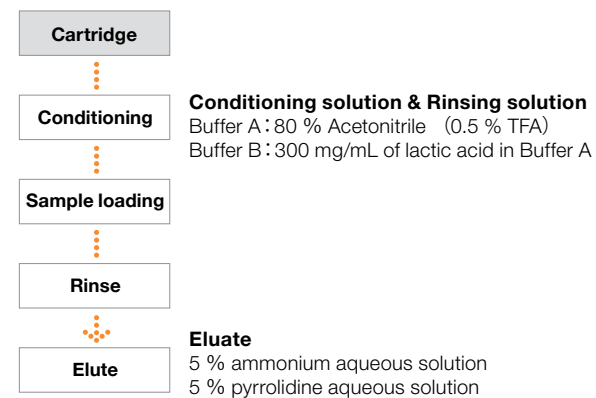


Titansphere Phos-TiO 50 mg/3 mL and 100 mg/3 mL spin columns

Typical Operating Conditions

Phos-TiO columns are intended for use with a desktop or other centrifuge.

While some of the versions of Phos-TiO are resemble pipette tips or SPE cartridges, these products are not intended for use with pipettes or SPE vacuum manifolds; the column internal configuration and particle size of the TiO beads requires centrifugal elution of all solutions.



Purified & enriched phosphopeptides

*MonoSpin C18 is recommended for desalting.

Specifications

Sample	Try (PO ₃ H ₂) - Angiotensin II	
Particle size	10 µm	
Cartridge	50 mg/3 mL	100 mg/3 mL
Binding Capacity	50 µg	100 µg

* The maximum sample loading volume depends on the matrix composition, concentration, freedom from particulates, and viscosity.

Titansphere™ Phos-TiO Kit Part Numbers

Description	Column Size	Quantity	Cat.No.
Titansphere Phos-TiO Kit	1 mg/10 µL	24 times	5010-21309
		96 times	5010-21310
	3 mg/200 µL	24 times	5010-21311
		96 times	5010-21312

Titansphere™ Phos-TiO Column Part Numbers

Description	Volume	Quantity	Cat.No.
Titansphere Phos-TiO Tip	10 µL	24 pcs	5010-21302
		96 pcs	5010-21303
	200 µL	24 pcs	5010-21307
		96 pcs	5010-21308

Description	Column Size	Qty(packaged unit)	Cat.No.
Titansphere Phos-TiO	50 mg/3 mL	25 (1 pcs)	5010-21290
	100 mg/3 mL	25 (1 pcs)	5010-21291

Description	Volume	Qty(packaged unit)	Cat.No.
Lactic Acid for Titansphere Phos-TiO	15 mL	1 pcs	5010-21295

Ordering Information for Reusable Adaptors which are used to mount Phos-TiO spin columns in collection tubes or 96-well plates.

Description	Quantity	Cat.No.
Centrifuge Adapter (10 µL, 200 µLtips)	24 pcs	5010-21514
96well plate centrifuge adapter for 10 µL Tips	1 pcs	5010-21340
	2 pcs	5010-21342
96well plate centrifuge adapter for 200 µL Tips	1 pcs	5010-21341
	2 pcs	5010-21343

96 well plate adapter is compatible with SBS standard plates.

