

For the Selective Purification & Enrichment of Phosphopeptides

Titansphere® Phos-TiO Kit

Introduction

Protein phosphorylation is one of the most important post-translational modifications for intracellular signal transduction. Therefore, the identification of phosphoproteins and the phosphorylated sites is important research issue.

However, it is difficult to detect phosphoproteins by mass spectrometry analysis due to their trace level of abundance and hard ionization efficiency. Therefore, the selective purification and enrichment of phosphopeptide become inevitable.

Currently, methods such as IMAC and metal oxide affinity chromatography are widely used to enrich phosphopeptides as these methods have high affinity to phosphopeptides. However, the enrichment efficiency in these methods depends on pH, the content of organic solvents, additives to the sample solvents, the sample loading speed and so on.

Therefore, we have developed a new kit, Titansphere Phos-TiO Kit to enable anyone to purify and enrich phosphopeptides with a high efficiency and a high reproducibility easily. The **titania (titanium dioxide)** particles are evenly formed in the tip.

Furthermore, we optimized the surface activity of titania particle of the Titansphere Phos-TiO Kit by the use of our state-of-the-art technologies.

Also in order to reduce the non-specific adsorption, we added the enhancer to the Titansphere Phos-TiO Kit. The enhancer contributes to the selective purification and enrichment.



Product Overview



Tip Columns

In Titansphere Phos-TiO Kit, there are two types of tip columns. One is uniformly filled with titania of 3mg to the Tip for 200uL. Another one is filled with titania of 1mg to the Tip for 10uL.

Please choose the most suitable type depending on the sample volume.

Features

Easy to Operate

The total number of process is only 4 steps.

And the operation time is only 40 minutes

High Capacity

Optimized the surface activity of Titansphere TiO particles to purify and enrich the phosphopeptides.

Procedure

Application

References

High Selectivity

An enhancer is added to reduce the non-specific adsorption and selectively purifies and enriches phosphopeptides.

High Throughput

Requires 2 types of centrifuge adaptors (sold separately) during the operation. Small number of samples or large number of samples using a 96-well format can be operated at the same time.

Titansphere® Phos-TiO Kit

NOTE	Description	Cat. No.
When placing an order for a Titansphere Phos-TiO Kit, a centrifuge adaptor also has to be ordered (unless you have one)	Tip column 1mg/10uL (24 pcs) Sol.A 2mL, Sol.B 2mL, Collection Tubes & Waste Tubes	1403024
	Tip column 1mg/10uL (96 pcs) Sol.A 6mL, Sol.B 6mL, Collection Tubes & Waste Tubes	1403096
	ip column 3mg/200uL (24 pcs) Sol.A 2mL, Sol.B 2mL, Collection Tubes & Waste Tube	1404024
	ip column 3mg/200uL (96 pcs) Sol.A 6mL, Sol.B 6mL, Collection Tubes & Waste Tube	1404096

Centrifuge Adaptor

Description	QTY	Cat. No.
Centrifuge Adaptor	24 pcs	5010-21514
96WP Centrifuge Adaptor for 10 μ L Tip	1 pcs	5010-21340
	2 pcs	5010-21342
96WP Centrifuge Adaptor for 200 μ L Tip	1 pcs	5010-21341
	2 pcs	5010-21343

Titania (titanium dioxide) Beads

Titansphere[®] TiO beads



◎ High Purity with Spherical Shape
It is easy for anyone to pack the bulks in tips and cartridges.

◎ Large Surface Area
Due to the large surface area, a high recovery can be obtained with a small amount of sample.

◎ Variety of Applications
As Titansphere TiO specially traps a part of phosphoric acid, there is a variety of applications, such as the enrichment of phosphopeptide, phosphorylated sugar, or Glyphosate etc.

Titansphere TiO beads

Description	Cat. No.
Titansphere [®] TiO 5 μ m Bulk Material 500 mg	1400B500
Titansphere [®] TiO 10 μ m Bulk Material 500 mg	1352B500

Procedure

Easy to Operate

The total number of process is only 4 steps. And the total operation time is only 40 minutes.

All the processes are performed by a centrifuge

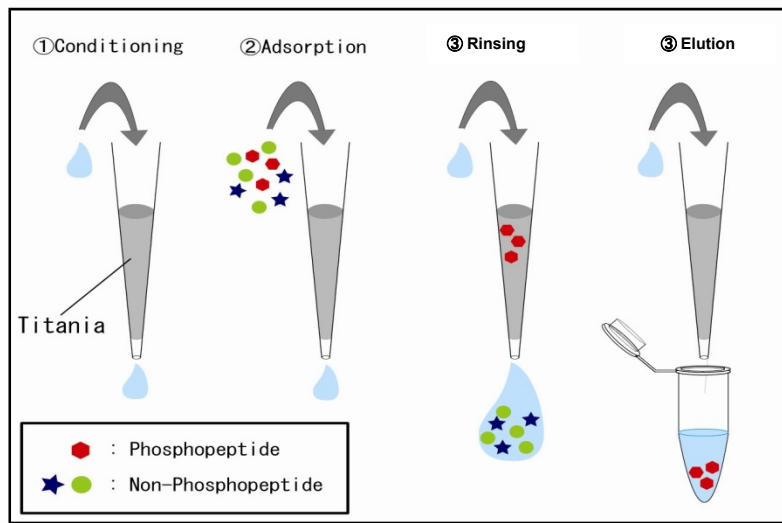
All the processes for purification and enrichment using "Titansphere Phos-TiO Kit" are by a centrifugation method. This enables to prevent any human error during the operation.

We have prepared 2 types of centrifuge adaptor, which are sold separately for Titansphere Phos-TiO Kit.

One is for small number of samples. It can be used by attaching to the collection tube and the waste tube.

The other type is for large number of samples. It is possible to insert to the 96-well format. The adaptor of 96-well format is compatible with the 96-well microplate (SBS standard).

Titansphere Phos-TiO Kit can be used various numbers of samples.



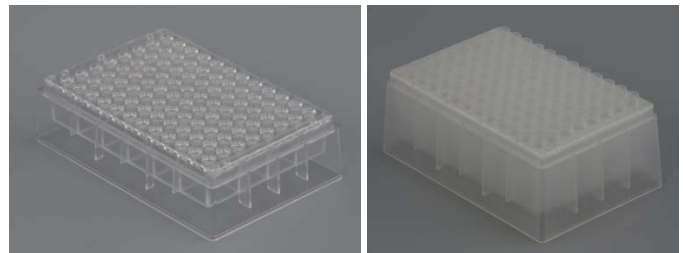
For small number of samples



Centrifuge Adaptor

How to Attach

For large number of samples



96-well format adaptor
for 10µL Tip

96-well format adaptor
for 200µL Tip

The 96-well format adaptor is compatible with the 96-well microplate (SBS standard)

Comparison of Recovery and Selectivity Between Five Commercially Available Phosphopeptide Enrichment Kits.

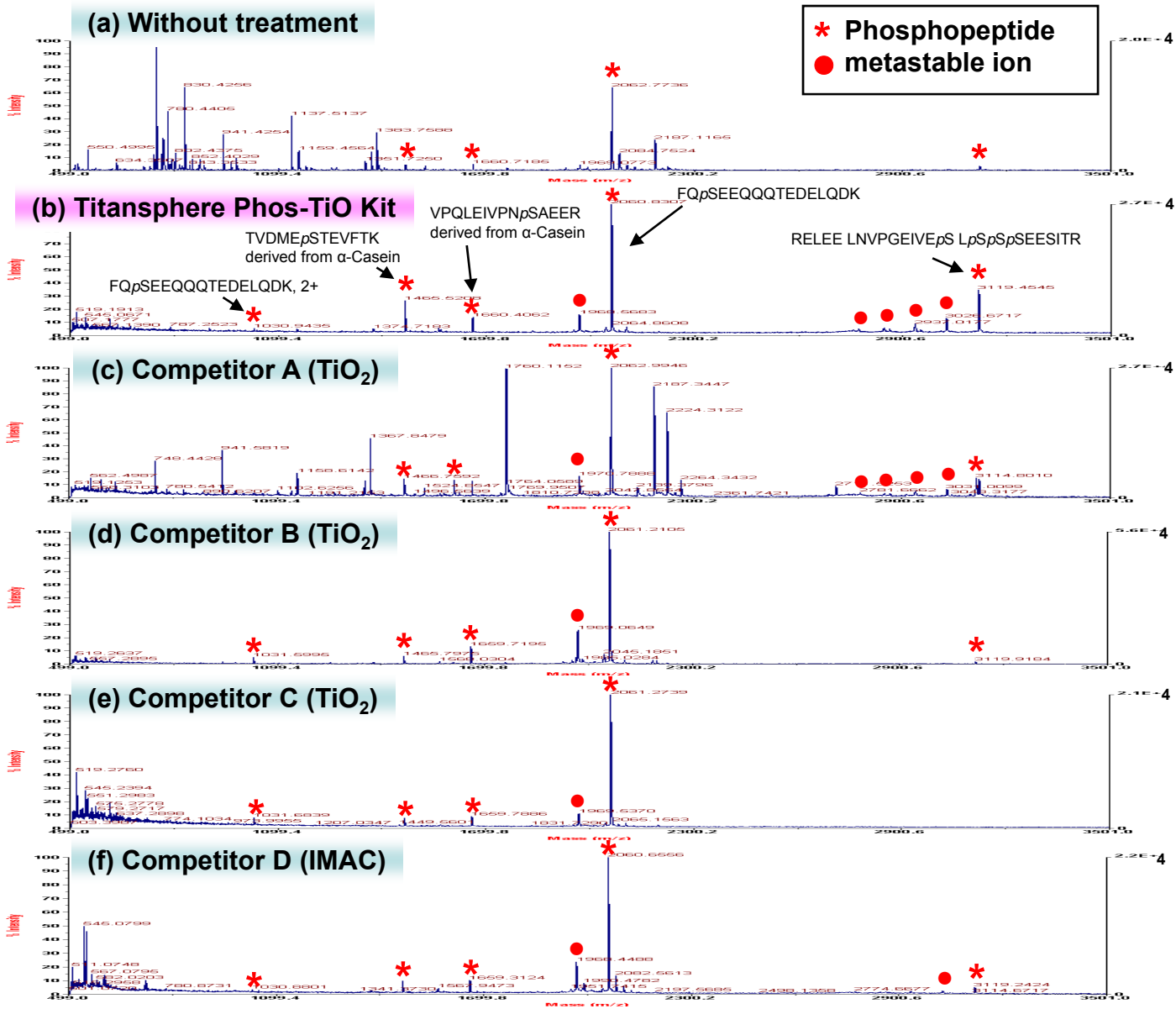


Figure 1. Comparison of commercial phosphopeptide enrichment kits by MALDI-TOF/MS.

Comparison of enrichment efficiency of tryptic digest of 2.5µg β-casein by MALDI-TOF/MS.

As shown above, phosphopeptides are selectively purified and enriched when using (b)Titansphere Phos-TiO Kit.

Even comparing the results from other competitors enrichment kits, Titansphere Phos-TiO Kit shows better sensitivity. In general, titania are known to catch the multi-phosphorylation site peptides (malti means over 4 sites). However, Titansphere Phos-TiO Kit showed higher sensitivity and detection for 4-phosphopeptides when compared from IMAC (f).

Comparison of Recovery and Selectivity between four Commercially Available Phosphopeptide Enrichment Kits.

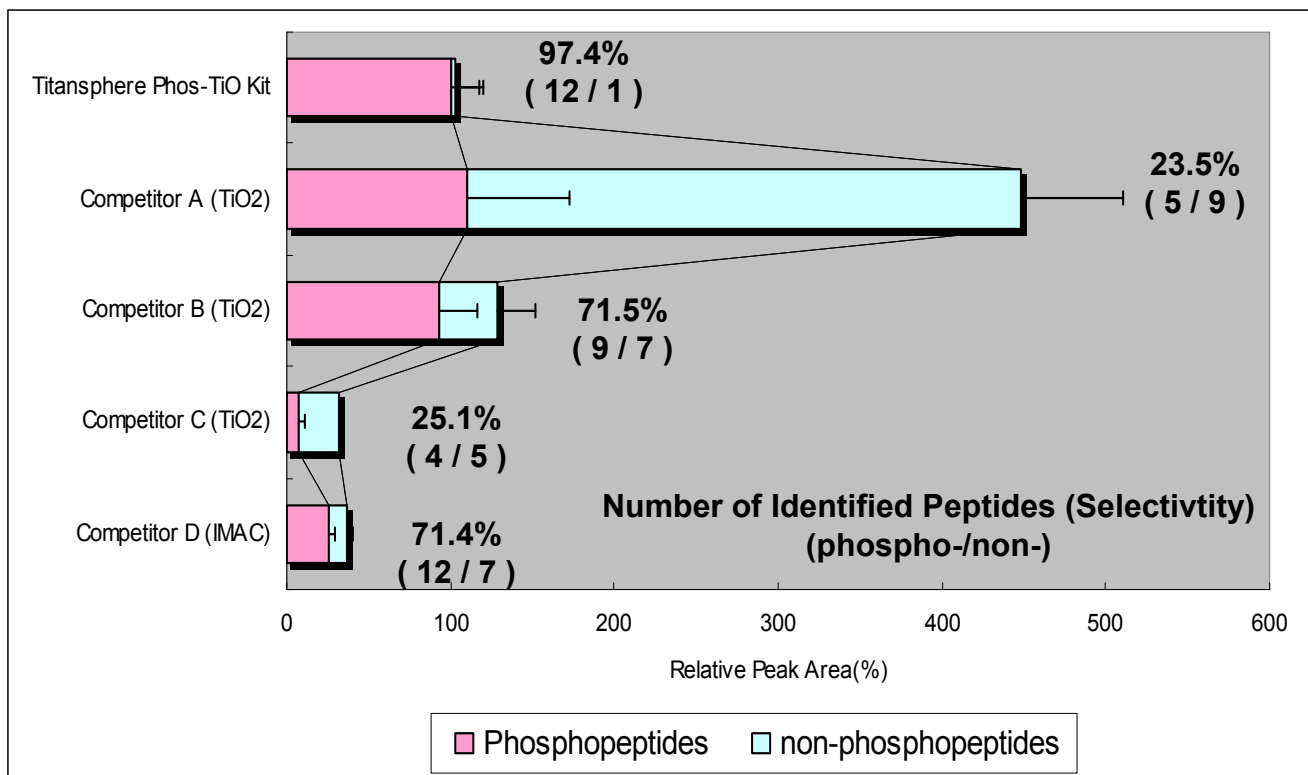


Figure 2. Comparison of commercially available phosphopeptide enrichment kits by LC-MS.

Comparison of Titansphere Phos-TiO Kit and IMAC from the Digest of HeLa Cell Lysate

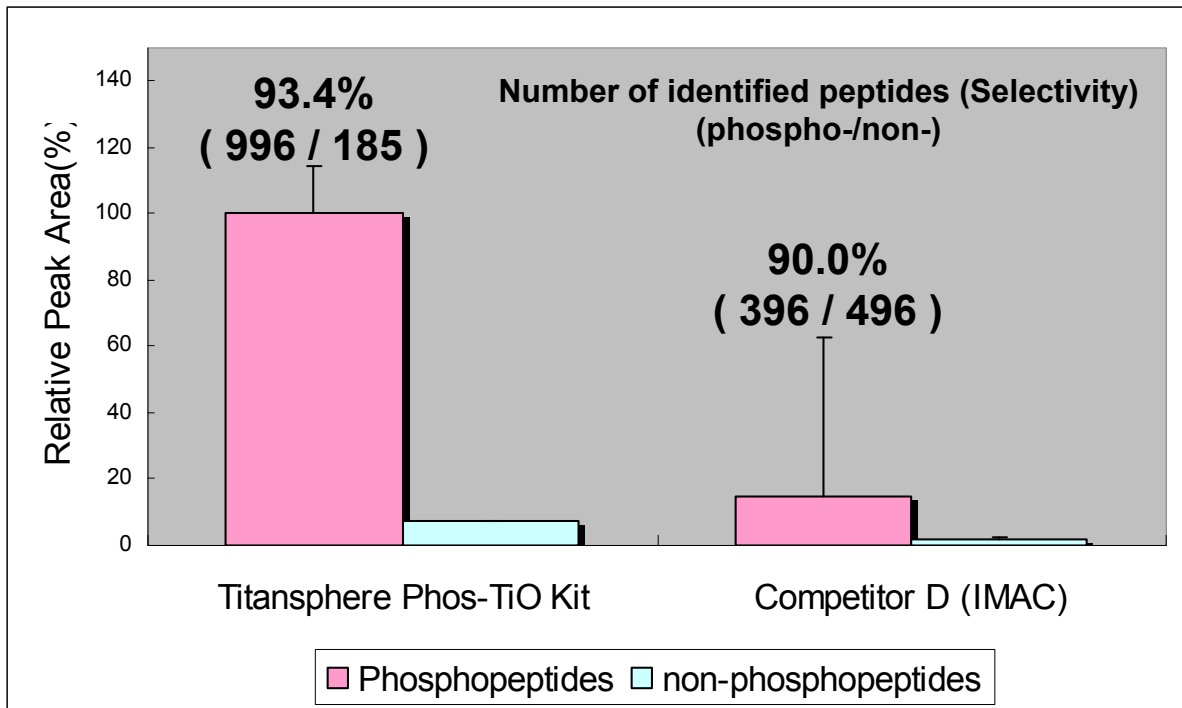


Figure 3. Comparison of recovery and selectivity of phosphopeptides identified from Tryptic digest of 100 μ g HeLa cell lysate by LC-MS.

References

- (1) Optimizing a selective enrichment conditions for phosphopeptides from tryptic digest of peptides & a quality comparison of titania column and IMAC column.

Larsen, et al.

Highly selective enrichment of phosphorylated peptides from peptide mixtures using titanium dioxide microcolumns.

Molecular & Cellular Proteomics 2005; 4: 873-886.

- (2) Verifying the dynamic of phosphorylation strength & time lapse of EGF-stimulated intracellular signaling factor (ex: GTPase, transcription factor, kinase...etc) using titania.

Olsen, et al.

Global, In Vivo, and Site-Specific Phosphorylation Dynamics in Signaling Networks.

Cell 127, 635-648, November 3, 2006.

- (3) Verifying a highly selective purification & enrichment method for phosphopeptides from tryptic digest of Hela cell lysate.

Sugiyama, et al.

Phosphopeptide Enrichment by Aliphatic Hydroxy Acid-Modified Metal Oxide Chromatography for NanoLC-MS/MS in Proteomics Applications.

Molecular & Cellular Proteomics 2007; 6: 1103-1109.

- (4) The influence of samples containing a surface active agent or denaturing agent (ex: SDS, urea...etc) using titania for phosphopeptide enrichment.

Jensen, et al.

Evaluation of the impact of some experimental procedures on different phosphopeptide enrichment techniques.

Rapid Commun. Mass Spectrom. 2007; 21: 3635-3645

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