Titansphere® Phos-TiO Kit

For the Selective Enrichment of Phosphopeptide

Introduction

Protein Phosphorylation is one of the most important post-translational modifications on a variety of cellular functions including cell proliferation. Therefore, the identification of Phosphoproteins and the Phophorylated sites is an important research issue.

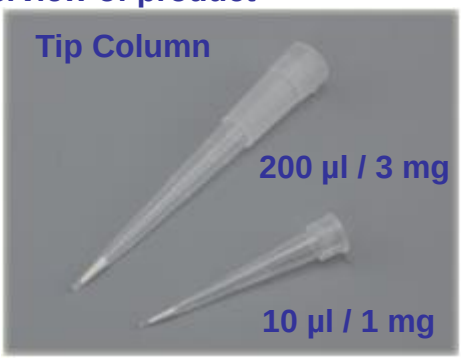
Unfortunately, it is difficult to detect Phosphoprotein by mass spectrometry because it hardly exists in cells and is difficult to ionize. To overcome this, selective enrichment of the Phosphopeptide is necessary.

Currently, sample preparation methods such as IMAC and metal oxide chromatography are widely used to enrich Phosphopeptides. These methods have high affinity to Phosphopeptides. However, they are difficult to optimize because the enrichment efficiency of these methods depends for example on the pH, the content of organic solvents, additives to the sample solvents, the sample loading speed etc.

For this reason, a new method is developed based on “Titansphere Phos-TiO tip-columns”. The **Titania (Titanium Dioxide)** particle is evenly formed in the tip column. In order to reduce the non-specific adsorption, an enhancer is added to the Titansphere Phos-TiO material. By adding the enhancer, it became possible to selectively purify and enrich the Phosphopeptide. **The total operation time is just 40 minutes and consist only about 4 steps.**

The Titansphere Phos-TiO Kit is to enable for everyone to purify and enrich Phosphopeptides, simple, fast and with a steady reproducibility.

Overview of product



In Titansphere Phos-TiO Kit, there are two types of tips. One is filled with Titania of 3mg to the Tip for 200µl. Another one is filled with Titania of 1mg to the Tip for 10µl. Please choose the suitable type depending on the sample volume.

Features

Easy to operate
The total operation is only 4 steps.
And the total operation time is only 40 minutes.

High adsorptive capacity
Optimized the surface activity of
Titansphere TiO particles improved the purifying and enrichment ability.

High Selectivity
An enhancer is added to reduce the non-specific adsorption and selectively purify and enrich the phosphopeptide.

Wide Number of Treatable Samples
Small number of samples or large number of samples using a 96-well format can be operated at the same time by a centrifuge method.

Titansphere® Phos-TiO Kit

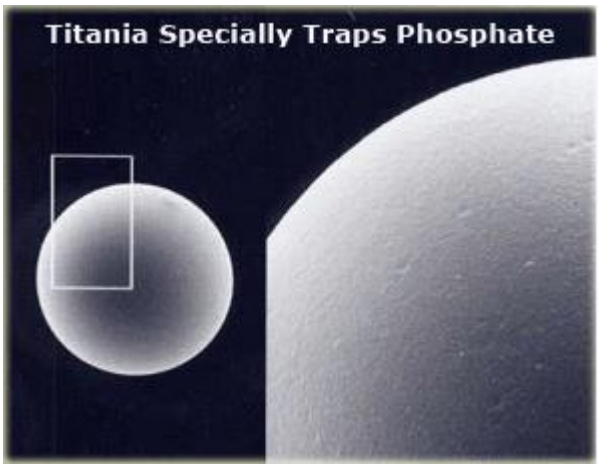
Name	Detail	Cat.No.	Price
Titansphere Phos-TiO Kit	TitanspherePhos-TiO Kit Tip column 1mg/10μL (24 pcs)	5010-21309	€ 150,00

Centrifuge adaptor

Name	Quantity	Cat.No.	Price
Centrifuge adaptor	24 pcs	5010-21514	€ 53,00
96WP adaptor for 10μL Tip	1 pcs	5010-21340	€ 64,00
	2 pcs	5010-21342	€ 129,00
96WP adaptor for 200μL Tip	1 pcs	5010-21341	€ 83,00
	2 pcs	5010-21343	€ 154,00

Titania (Titanium Dioxide) beads

Titansphere® TiO beads



◎ Pure Spherical Shape
It is easy for anyone to pack the beads materials to narrow places such as a tip head.

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

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

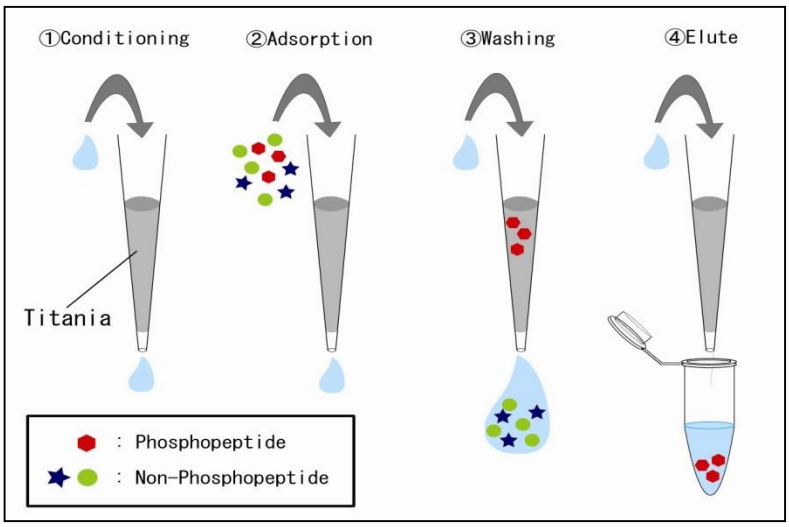
Titansphere TiO beads

Items	Cat. No.
Titansphere®TiO 5 μm Bulk Material 500 mg	5020-75000
Titansphere®TiO 10 μm Bulk Material 500 mg	5020-75010
Empore™ DISK C8 Diameter 47 mm、Thickness 0.5 mm、20 pcs	5010-30002
Empore™ DISK C8 Diameter 90 mm、Thickness 0.5 mm、10 pcs	5010-30003

Procedure

Easy to Operate

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



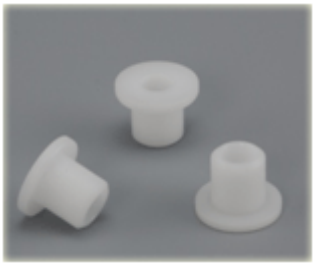
Centrifuge Operation

All the operations of “Titansphere Phos-TiO Kit" are done using centrifugation method.

We have prepared 2 types of centrifuge adaptors, which are sold separately for Titansphere Phos-TiO Kit.
One is for small number of samples. It can be used by attaching to the centrifuge tube. Another is for large number of samples. It is possible to insert the tip column to the 96-well format as much as you need. The adaptor of 96-well format is compatible with the 96-well microplate (SBS standard).

Titansphere Phos-TiO Kit can be used for various numerical samples.

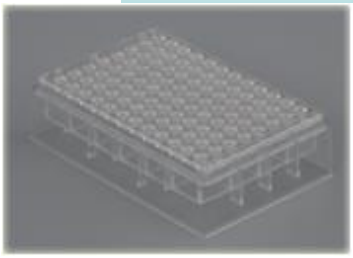
For small number of samples



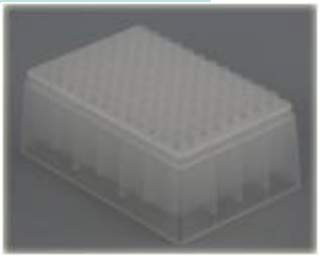
Centrifuge Adaptor

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)

Application

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

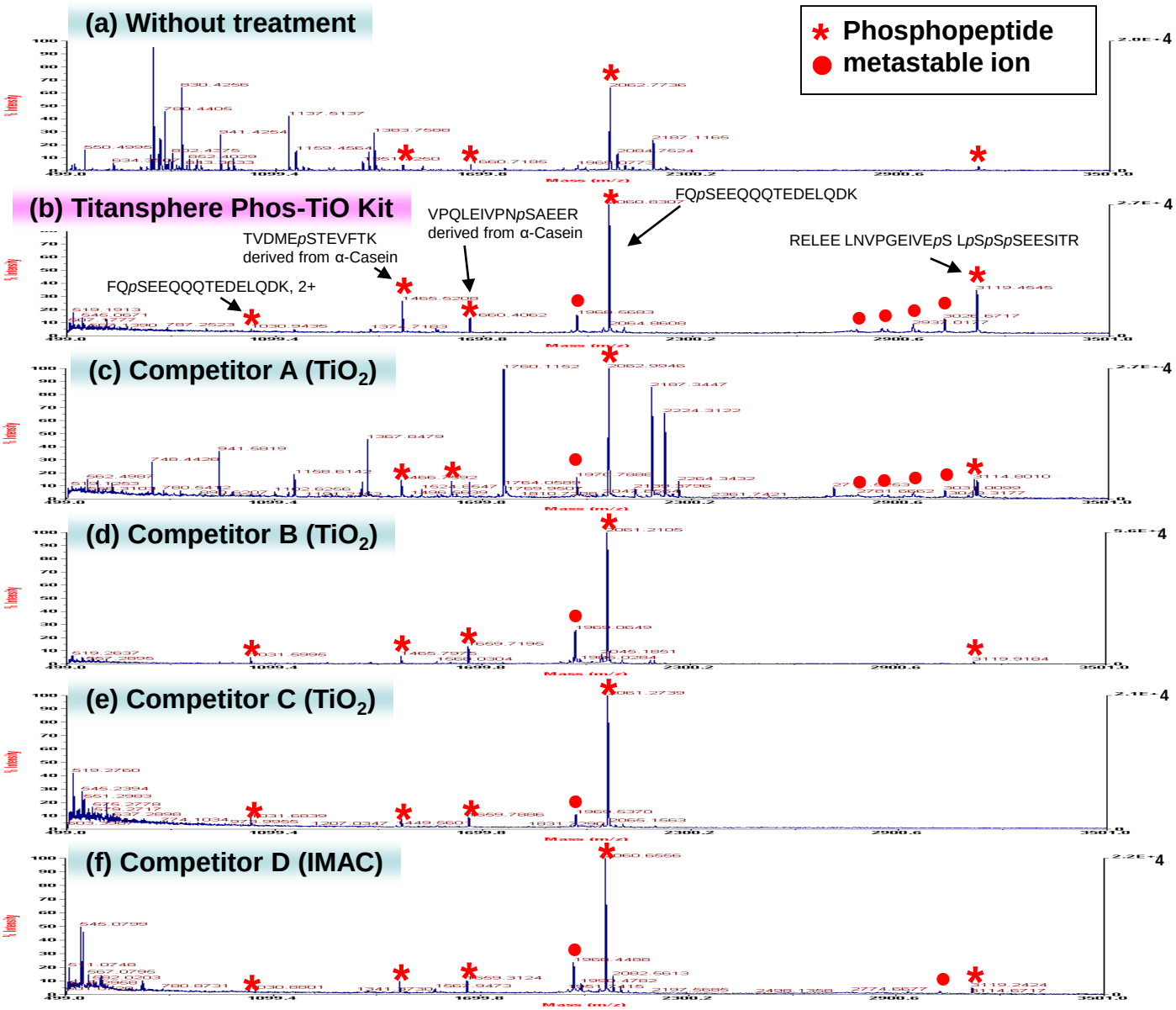


Figure 1. Comparison of Enrichment Efficiency of Tryptic Digest of 2.5µg β-casein by MALDI-TOF/MS

As shown above, Phosphopeptides are selectively purified and enriched when using (b)Titansphere Phos-TiO Kit. Titansphere Phos-TiO Kit shows better sensitivity than competitors. In general, Titansphere® TiO is known to catch the Multi-Phospholylation site peptides (multi means over 4 sites). However, Titansphere Phos-TiO Kit showed higher sensitivity and detection for 4-Phosphopeptides when compared to IMAC (f).

Comparison of Recovery and Selectivity Among 4 Commercially Available Phosphopeptide Enrichment Kits

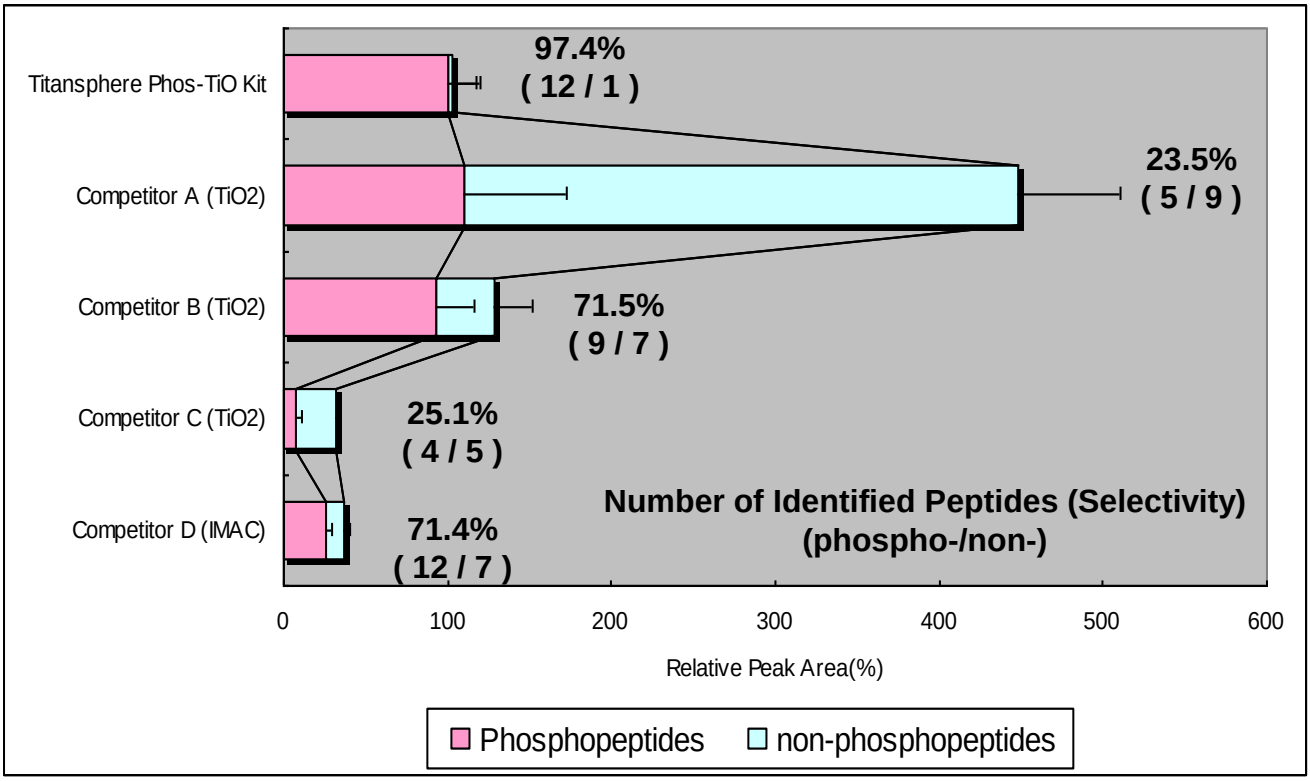


Figure 2. Comparison of Commercially Available Phosphopeptide Enrichment Kits by LC-MS.

Each 2.5 µg of Tryptic digest of α-Casein, Fetuin, Phosvitin was used to compare the selectivity and recovery of Phosphopeptides among commercially available kits (n=3).

The % in the graph above shows the phosphopeptides peak area ratio within all the detected peptides peak area.

Using Titansphere Phos-TiO Kit, 97.4% Phosphopeptides peak area rate were obtained. Compared to the Titansphere Phos-TiO Kit peak area, competitor A showed 23.5%, 71.5% for competitor B, 25.1% for competitor C, and competitor D (IMAC) was 71.4%. Although Competitor A showed the largest Phosphopeptides peak area, an extremely large Non-Phosphopeptides peak area was detected. Titansphere Phos-TiO Kit showed the highest selectivity.

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

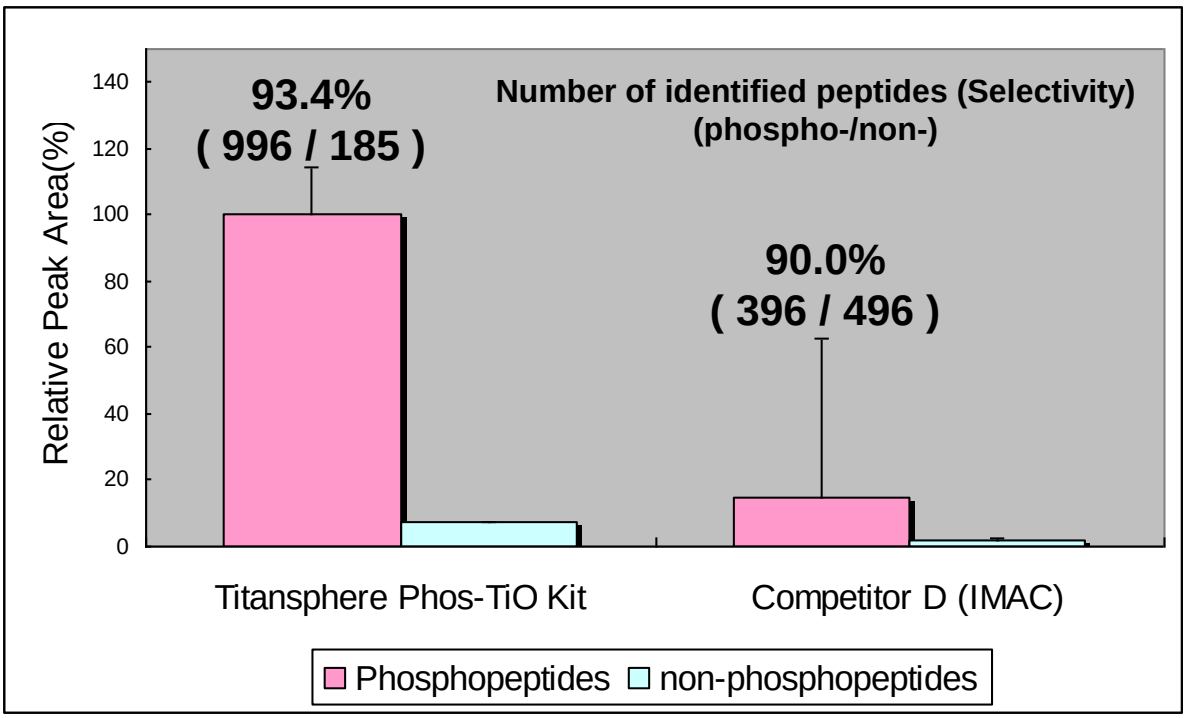


Figure 3. Comparison of Recovery and Selectivity of Phosphopeptides Identified from Tryptic Digest of 100µg Hela Cell Lysate by LC-MS.

Tryptic digest of 100ug HeLa cell lysate was used to compare the enrichment efficiency between Titansphere Phos-TiO Kit and IMAC by LC-MS (n=3).

Titasphere Phos-TiO Kit detected 6.7 folds more of the Phosphopeptides peak area than IMAC. The Selectivity of Titansphere Phos-TiO Kit and IMAC were more than over 90%. However, Titansphere Phos-TiO Kit identified 996 Phosphopeptides and IMAC 396. Recovery is superior in Titansphere Phos-TiO Kit.

The “%” shows the detected Phosphopeptides peak area rate against total peptides peak area.

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

Prices for 2016

GL Sciences B.V.
De Sleutel 9
5652AS Eindhoven
The Netherlands
info@glsciences.eu
www.glsciences.eu