

For the Selective Enrichment of Phosphopeptide

# Titansphere<sup>®</sup> Phos-TiO Kit



Overview of Product



Tip Column

In Titansphere Phos-TiO Kit, there are two types of tip columns. One is 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 suitable type depending on the sample volume.

## Introduction

Protein phosphorylation is one of the extremely important posttranslational modifications on a variety of cellular functions including cell proliferation. Therefore, the identification of phosphoproteins and the phosphorylated sites is an important research issue.

However, it is difficult to detect phosphoprotein by mass spectrometry as it hardly exists in cells and is difficult to be ionized. Therefore, the selective enrichment of phosphopeptide becomes necessary.

Currently, methods such as IMAC and metal oxide 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 for anyone to purify and enrich phosphopeptides with a steady reproducibility. The **titania (titanium dioxide)** particle is evenly formed in the tip column.

Furthermore, we optimized the surface activity of titania particle with the Titansphere Phos-TiO Kit to purify and enrich phosphopeptides.

Also in order to reduce the non-specific adsorption, we added the enhancer to the Titansphere Phos-TiO Kit. By adding the enhancer, it became possible to selectively purify and enrich the phosphopeptide.



## Feature

### Easy to Operate

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

### High Selectivity

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

### High Adsorptive Capacity

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

### 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<sup>®</sup> Phos-TiO Kit

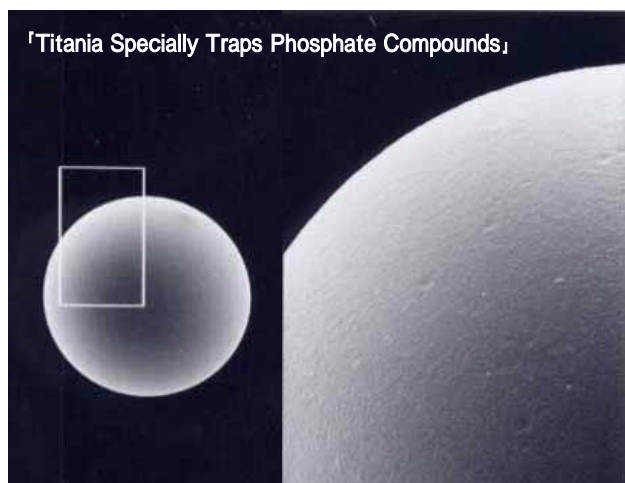
Name	detail	Cat.No.
Titansphere Phos-TiO Kit	Tip column 1mg/10uL (24 pcs) Sol.A 2mL, Sol.B 2mL	5010-21300
	Tip column 1mg/10uL (96 pcs) Sol.A 6mL, Sol.B 6mL	5010-21301
	Tip column 3mg/200uL (24 pcs) Sol.A 2mL, Sol.B 2mL	5010-21305
	Tip column 3mg/200uL (96 pcs) Sol.A 6mL, Sol.B 6mL	5010-21306

# Centrifuge adaptor

Name	QTY	Cat.No.
Centrifuge adaptor	24 pcs	5010-21514
96WP adaptor for 10 $\mu$ L Tip	1 pcs	5010-21340
	2 pcs	5010-21342
96WP adaptor for 200 $\mu$ L Tip	1 pcs	5010-21341
	2 pcs	5010-21343

## Titania (titanium dioxide) Beads

# Titansphere<sup>®</sup> 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 <sup>®</sup> TiO 5 $\mu$ m Bulk Material 500 mg	5020-75000
Titansphere <sup>®</sup> TiO 10 $\mu$ m Bulk Material 500 mg	5020-75010
Empore <sup>™</sup> DISK C8 Diameter 47 mm, Thickness 0.5 mm, 20 pcs	5010-30002
Empore <sup>™</sup> DISK C8 Diameter 90 mm, Thickness 0.5 mm, 10 pcs	5010-30003

## Easy to Operate

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

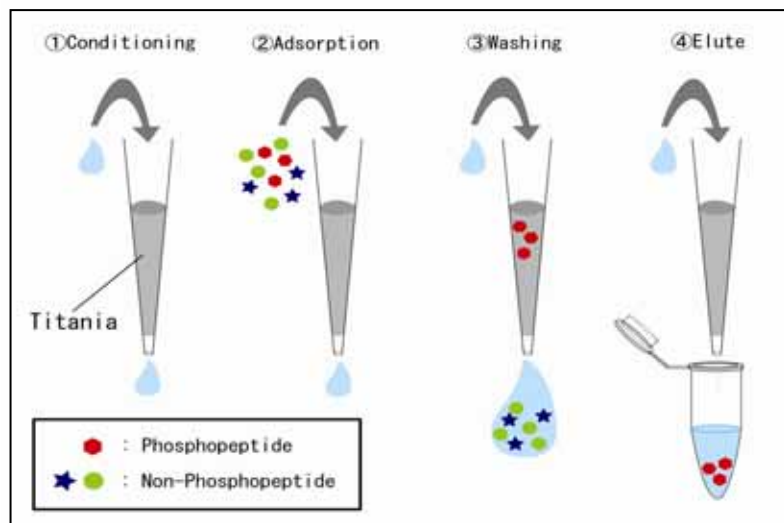
## Centrifuge Operation

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

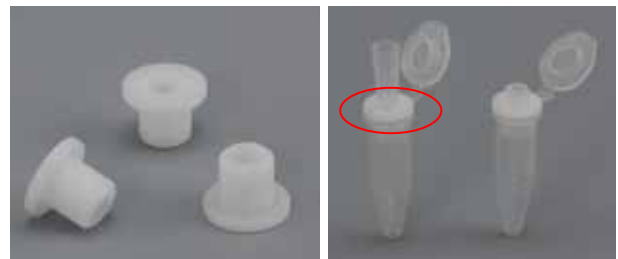
We have prepared 2 types of centrifuge adaptors, which are sold separately for Titansphere Phos-TiO Kit.

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.



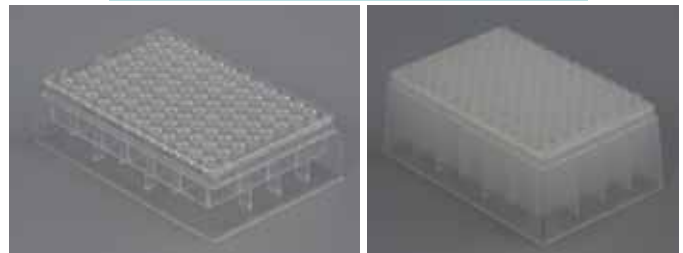
### For small number of samples



Centrifuge Adaptor

How to Attach

### For large number of samples

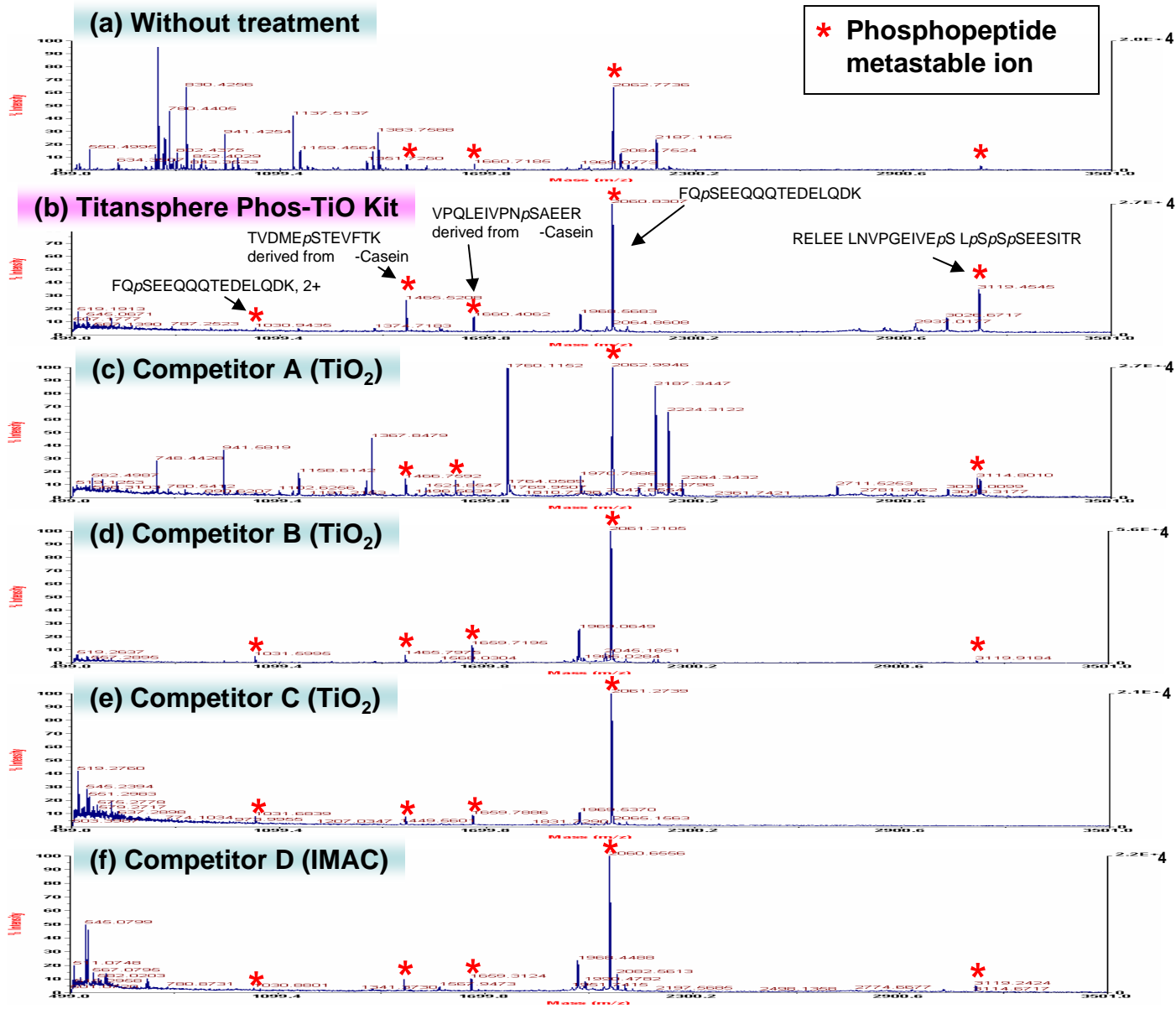


96-well format adaptor  
for 10 µ L Tip

96-well format adaptor  
for 200 µ L 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.**

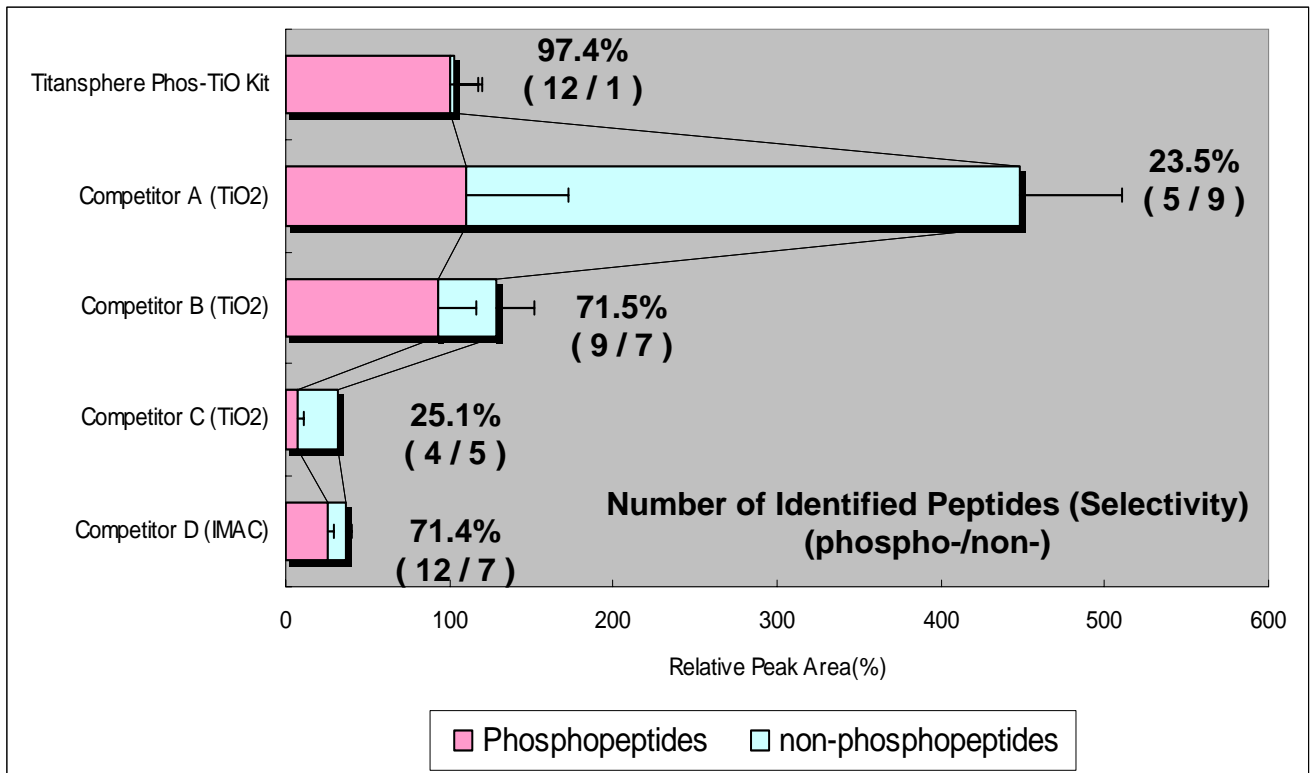
Comparison of enrichment efficiency of tryptic digest of 2.5 μg  $\alpha$ -casein by MALDI-TOF/MS.

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 is known to catch the multi-phosphorylation 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).

Metastable ion is dephosphorylation by collision cell

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

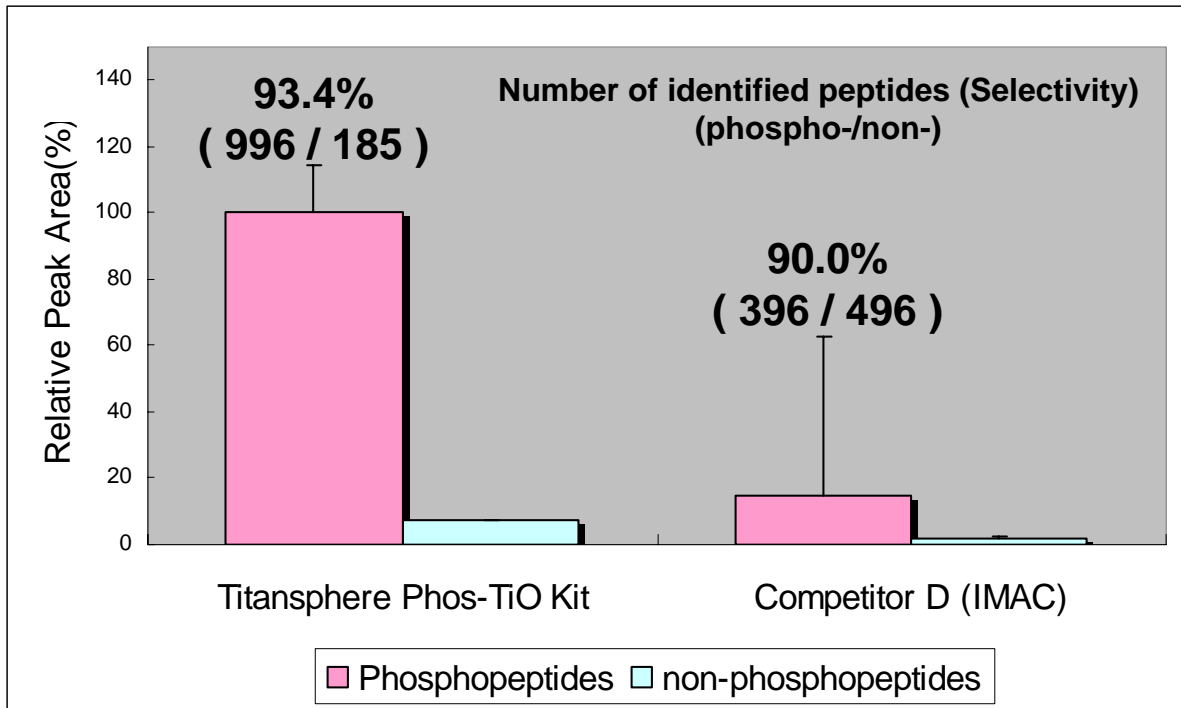


**Figure 2. Comparison of Commercially Available Phosphopeptide Enrichment Kits by LC-MS.**

Each 2.5 μg of Tryptic digest of  $\beta$ -Casein, Fetuin, Phosvitin were used to compare the enrichment selectivity and recovery among commercially available phosphopeptide enrichment kits (n=3).

Using Titansphere Phos-TiO Kit, 97.5% phosphopeptides peak area rate were obtained. Compared to the Titansphere Phos-TiO Kit peak area, competitor A showed 110%, competitor B was 92.6%, competitor C was 7.9%, and competitor D (IMAC) was 25.9%. 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 100 µg 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).

Titansphere 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.

The figures in “( / )” show the number of identified peptides (phospho- / non-phospho-).



- (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