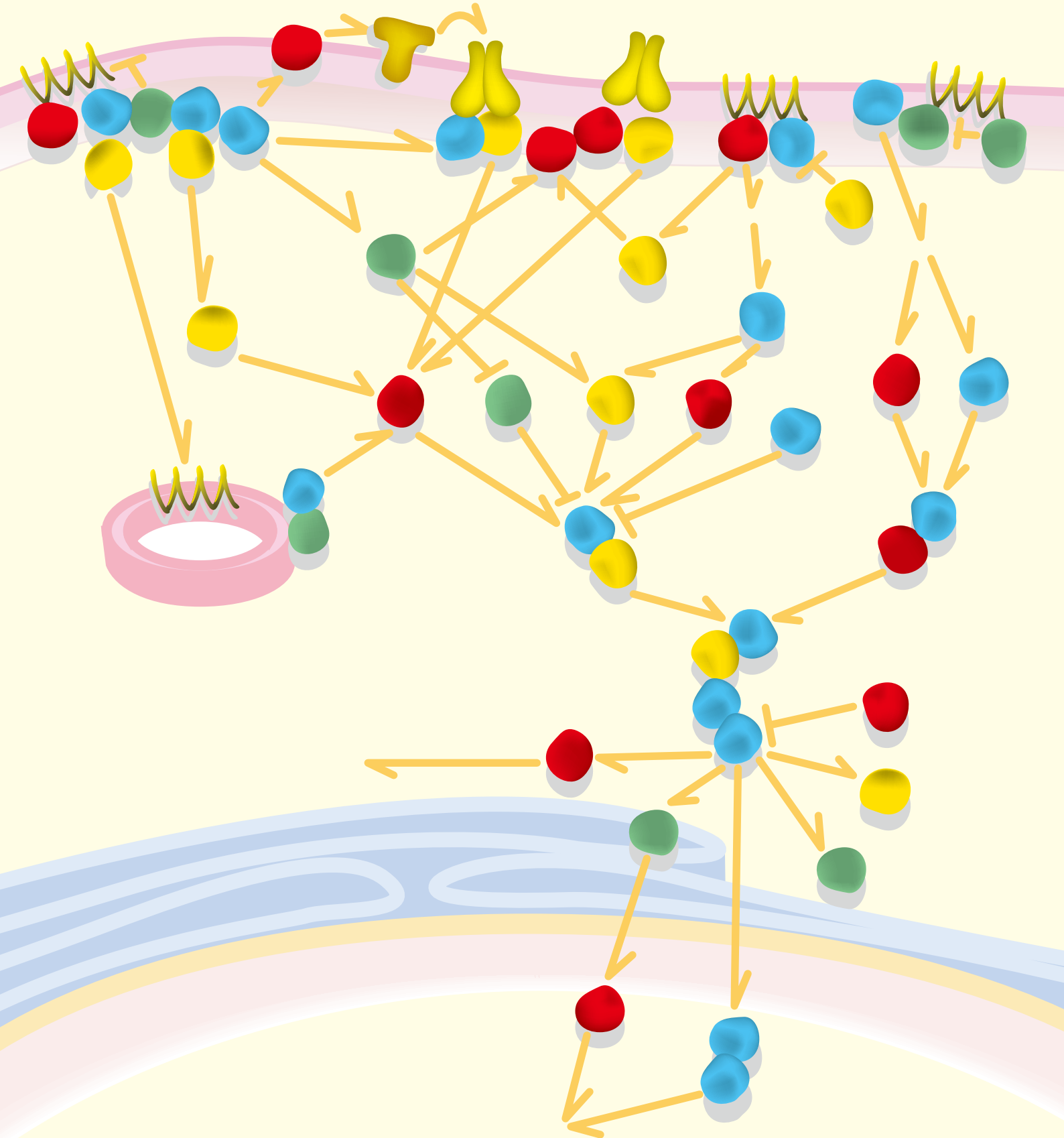


Phosphopeptide Purifying & Enrichment Products



Importance of Phosphopeptide Research

Researching the reversible phosphorylation plays a very important role to know the cell regulation, such as the cell cycles, cell growths, differentiation, and apoptosis.

However, when the phosphopeptides in proteins are analyzed as an indicator, it is hard to detect them as the amount of phosphopeptide and its ionization efficiency are extremely low.

Therefore, it is critical to enrich and purify only phosphopeptides from the protein digest.

For Those Who Research *Signalization*

How strong is the phosphopeptide of each signaling factor after stimulating the cells?
How can you find the factors that emerge on the same time-course?

Here is the solution.

With uniform and spherical Titanium Dioxide beads, "Titansphere® TiO", it is easy to learn the **phosphorylation dynamics in the cell lysate** by a simple centrifuge sample preparation procedure.

Problems

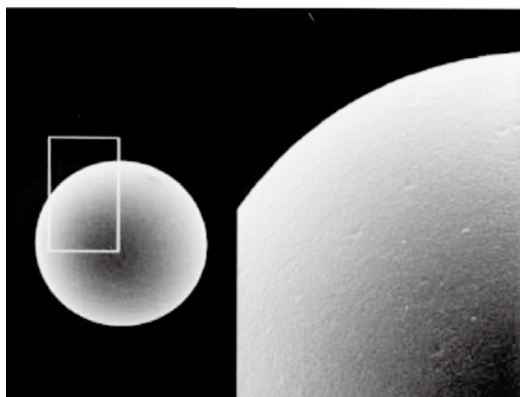
A Very Little Phosphoprotein in Cells

Too Many Spots That Become Phosphorylated

Low Ionization Efficiency

For Selective Purification and Enrichment of Phosphopeptides

Titansphere® TiO Beads



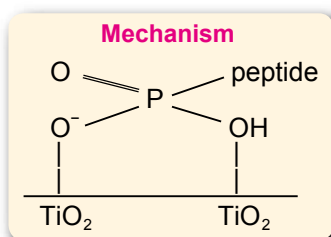
Evenly spherical shaped porous particle, Titansphere® TiO (Titanium Dioxide: TiO_2), is highly effective bead to selectively enrich and purify phosphopeptides.

Numbers of lucubrations have been proving that the Titansphere® TiO has the efficient and selective enrichment property of phosphopeptides.

Please try the satisfying performance and stability of Titanium bead that is best optimized to purify and enrich phosphopeptides.

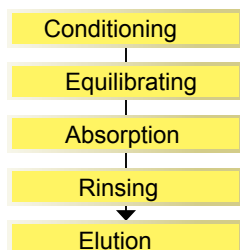
■ Principle

Phosphate groups are absorbed on the surface of Titanium Dioxide under acidic condition



■ Purification & Enrichment Protocol

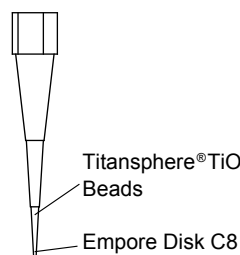
Operation takes only 5 minutes by centrifuge.



■ Specifications

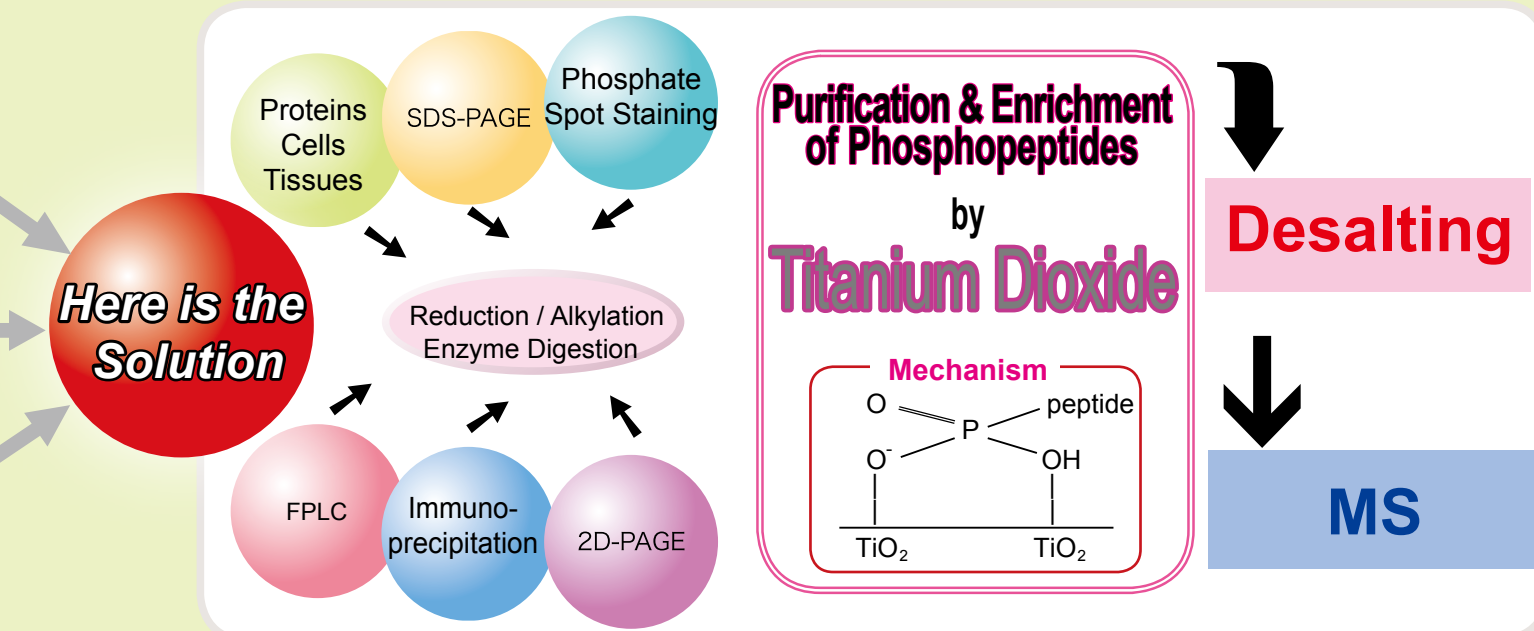
Particle Size	5µm, 10µm
Particle Shape	Spherical
Absorption Spot	Titanium Dioxide Crystal
Pore Size	100Å (10nm)
pH Range	2 ~ 12
Gravity	1.74

■ How to Make a Titanium Column

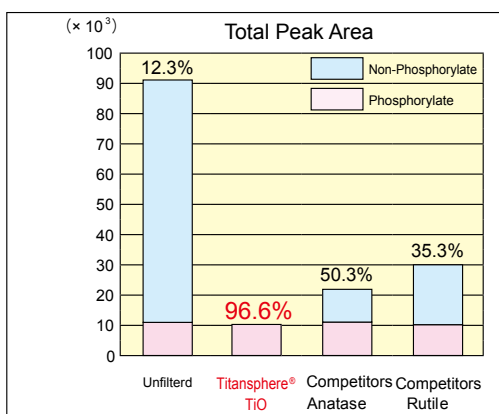


- Cut the Empore Disk C8 and put it at the tip of the 200ul pipette tip to be used as a filter
- Stir and suspend the Titansphere® TiO at $\text{CH}_3\text{CN}/\text{H}_2\text{O}=80/20$.v/v then load it to the tip (Adjust the amount of the beads depending on the purpose)
- Pressure the tip by a plastic syringe

Titansphere® TiO was invented based on the cooperation from Dr. Hiroshi Nakamura, Tokyo University of Science.



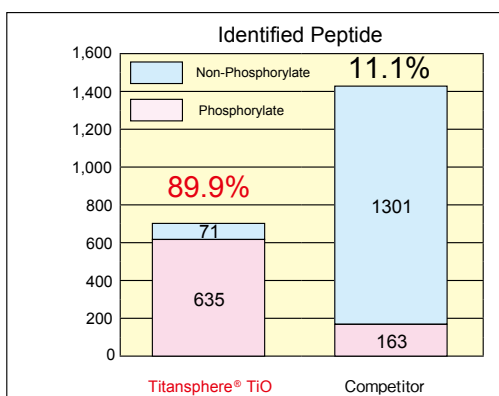
Application



Purification and Enrichment of Standard Proteins

The graph left shows the comparison data of Titansphere® TiO and competitors' Titanium Dioxide Beads. There are 2 kinds of crystal structures in Titanium Dioxide; Anatase and Rutile. Titansphere® TiO has the best optimized crystal structure to purify and enrich phosphopeptides. Detected phosphopeptides rate without filtering was 12.3% while with Titansphere® TiO it was 96.6%.

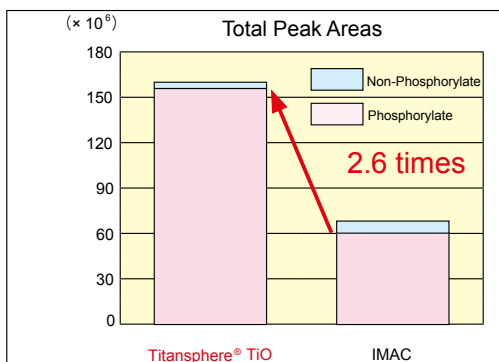
Sample : Tryptic digests of α -Casein
Fetuin and Phosvitin
Sample Volume : Standard Protein total 7.5 μ g
Used Titansphere® Bulk: 1 mg



Efficiency of the Purification from Hela Cell Lysate

The graph left shows the superior performance of Titansphere® TiO using the Hela Cell Lysate that contains abundant non-phospho related peptides. Comparing the number of the identified phosphopeptides with the competitor's product, Titansphere® TiO showed 89.9% even from bland cell lysate extract.

Sample : Hela Cell Lysate
Sample Volume : 50 μ g
Used Titansphere® Bulk : 1 mg



Comparison with IMAC

The graph left shows the performance comparison between Titansphere® TiO and IMAC using Arabidopsis cell extract. Titansphere® TiO showed 2.6 times of phosphopeptide peak area and 1.8 times of identified number of phosphopeptide compared to IMAC.

Sample : Arabidopsis Cell Extract
Sample Volume : 100 μ g
Used Titansphere® Bulk: 1 mg

* Identified Numbers of Phosphopeptide

Products	Phosphorylate	Non-Phosphorylate
Titansphere®TiO	846	198
IMAC	474	379

For the Selective Enrichment of Phosphopeptides

Titansphere® Phos-TiO Kit

With the constant amount and the best optimized condition of Titansphere® bulk packed in the tip column, Titansphere® Phos-TiO Kit offers an easier and stable operation to enrich phosphopeptides. Titansphere® Phos-TiO Kit comes with the special buffer and operation manual to achieve even better purification and enrichment of phosphopeptides than Titansphere® TiO beads.

Tip Column



There are 2 types of tip columns. One is filled with Titansphere® of 3mg in the 200nL tip column. The other one is filled with Titansphere® of 1mg in the 10uL tip column.

Product Overview



Features

● High Selectivity

Due to the high affinity against the phosphate peptides, phosphopeptides can be detected by MS (Mass Spectrometry) even from the bland cell lysate extract that contains a trace amount of phosphopeptides.

● Easy to Operate

Only 5 steps to go and takes just 40 minutes! All the steps can be done by centrifuge technique that allows littel humam error.

● Various Number of Treatable Samples

Each tip column is independent. With the special adaptor (separately available) any number of samples can be treated at once.

● Easy-to-Follow Manual

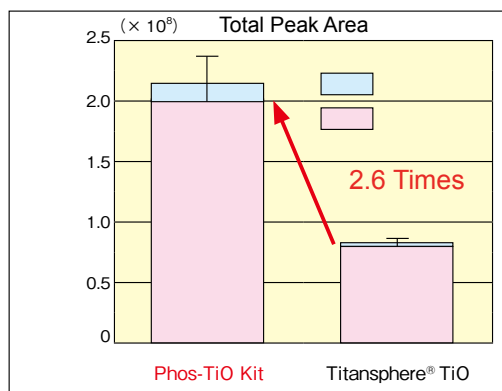
For any user's convenience, the operation manual comes with the Kit.

Phosphopeptides Loading Capacity

Sample	Tyr(PO ₃ H ₂)-Angiotensin II	
	3mg /200 μL	1mg /10 μL
Tip Column		
Loading Volume	3.5ug	1.2ug

■ Titansphere® Phos-TiO Kit Performance

High performance Titansphere® beads are employed for Pho-TiO Kit



For the better absorption of phosphopeptides, the existing Titansphere® beads was even improved. The absorption characteristic stays as ever and the absorption ability was improved. Compared to the existing Titansphere®, the Phos-TiO Kit showed **2.6 times** total peak area, of phosphopeptide and **1.6 times** numbers of identified phosphopeptides.

Sample : Hela Cell Lysate
 Sample Load Volume: 50ug
 Used Titansphere® Bulk : 1mg

* Identified Numbers of Phosphopeptide

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

Titansphere® Phos-TiO Kit was invented based on the cooperation from Dr. Yasushi Ishihama, Keio University.

■ **Titansphere® TiO and Phosphopeptide**

In April 2005, Dr. M. R. Larsen and his co-researchers published a paper stating that the use of Titansphere® TiO resulted in enriching highly selective phosphopeptide more than IMAC.

Using DHB (2.5 dihydroxybenzoic) acid as a washing buffer, they targeted the trypsin digest of standard proteins and present how to make full use of the characteristics of titanium dioxide more effectively in the paper.

This paper proves that a simple preparation without the separation or purification by electrocataphoresis or antibodies enables detection of phosphopeptide in relatively small amounts and has a low ionization efficiency by using a mass spectrometer (MS).

1. M. R. Larsen, T. E. Thingholm, O. N. Jensen, P. Roepstorff, and T. J. D. Jorgensen, "Highly selective enrichment of phosphorylated peptides from peptide mixtures using titanium dioxide microcolumns", *Molecular & Cellular Proteomics* 2005; 4: 873-886.

■ **Phosphopeptide in Cell Lysate and Titansphere® TiO**

In November 2006, Dr. Jesper V. Olsen and his co-researchers published a paper to state that phosphopeptide can be enriched from cell lysate by using Titansphere® TiO₂. Also in this paper, they illustrated the strength of phosphopeptide, which are signaling factors such as GTPase, transcription factor, and kinase etc., and the dynamic state on the same time-course in the cells that received EGF (Epidermal Growth Factor) stimulation by purifying with Titansphere® TiO.

This paper proves that the selective purification /enrichment of phosphopeptide by Titansphere® TiO can be applied in cells and also it is possible to determine the dynamic state of each factor.

2. Jesper V. Olsen, Blagoy Blagoev, Florian Gnad, Boris Macek, Chanchal Kumar, Peter Mortensen, and Matthias Mann. "Global, In Vivo, and Site-Specific Phosphorylation Dynamics in Signaling Networks" *Cell* 127, 635-648, November 3, 2006.

■ **High Selectivity of Titansphere® TiO**

In February 2007, Dr. Ishihama and his co-researchers proved that more concentrated phosphopeptide can be enriched by using lactic acid during the purification with Titansphere® TiO₄. Lactic acid made the LC-MS analysis easier and the detection sensitivity was improved.

In the paper, they stated that the detected peptide was all phosphopeptide with a standard protein. Moreover, they proved that phosphopeptide can be detected selectively from Hela cell lysate.

4. Naoyuki Sugiyama, Takeshi Masuda, Kosaku Shinoda, Akihiro Nakamura, Masaru Tomita, Yasushi Ishihama "Phosphopeptide Enrichment by Aliphatic Hydroxy Acid-Modified Metal Oxide Chromatography for NanoLC-MS/MS in Proteomics Applications" *Molecular & Cellular Proteomics* 2007; 6: 1103-1109.

Phosphopeptide HPLC Online Enrichment Column

Titansphere® TiO Enrichment Column



[Features]

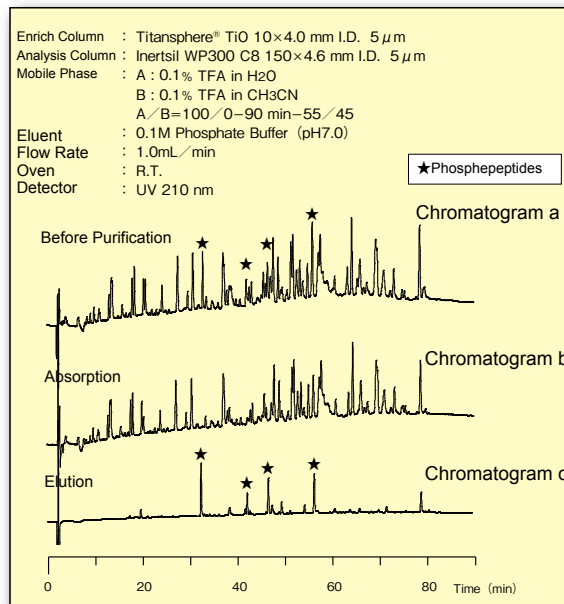
- Specifically Traps Phosphate Compounds
- Metal Free Column Available
- High Stability and Endurance

[Sample Load Volume]

Inner Diameter (I.D.mm)	Volume (μL)	Flow Rate (1mm/sec. μL/min.)	Sample Load Volume (1mm/sec. μg) (FW:about 1,000)
0.05	0.020	0.12	0.00075
0.1	0.079	0.47	0.003
0.2	0.314	1.89	0.012
0.3	0.707	4.26	0.021
1	7.850	50	0.302
1.5	17.663	100	0.679
2.1	34.619	200	1.331
3	70.650	400	2.718
4.6	166.106	1000	6.39

* Sample load volume is calculated based on reference literatures. When the line velocity becomes faster, the load volume will become smaller.

* Column Length: 10mm Particle Size: 5μm, Absorption Solvent: 0.1M Acetic Acid



* Column Switching Example of Trypsin Digestion with Casein Mixture

Non-Metal Phosphopeptides Extraction column

[Capillary EX-Nano Column (Without column performance report) Fused Silica Tubing and Peek Frits are used]

COLUMN SIZE (I.D.x Length)	Cat. No.
0.05 x 30mm or 50	5020-50Ni30-TiO
0.075 x 30mm or 50	5020-75Ni30-TiO
0.1 x 30mm or 50	5020-100Ni30-TiO2
0.2 x 30mm or 50	5020-200Ni30-TiO2

[Titansphere®, 5μm, Non-Metal, All Peek Column (Peek Frits are used)]

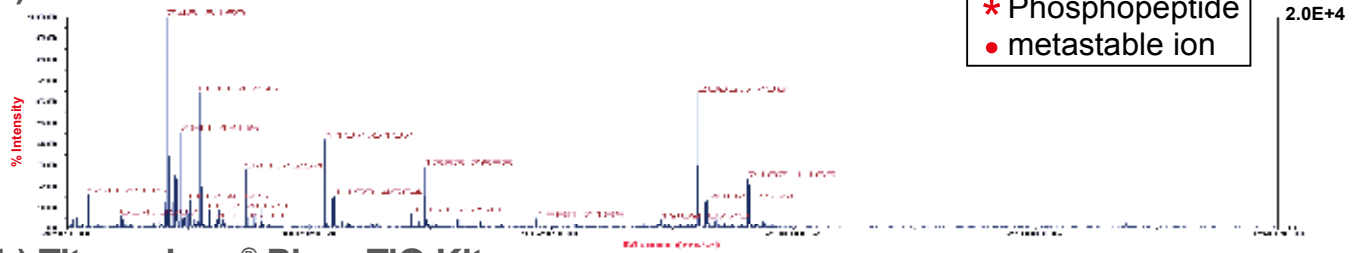
COLUMN SIZE (I.D.x Length)	Cat. No.
0.3 x 2mm All Peek column	5020-01879-5u-TiO2

[Titansphere®, 5μm, Non-Metal Cartridge Column Ei (Peek Frits are used)(10mm Cartridge Length)]

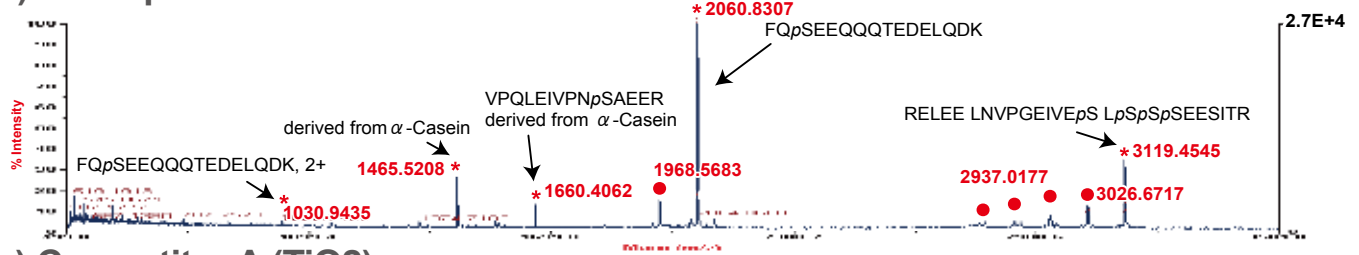
ITEM NAME	Cat. No.
Holder for Non-Metal Cart.	5020-08650
2 CARTRIDGES, 1.0 I.D.	5020-08683-5u-TiO2
2 CARTRIDGES, 2.1 I.D.	5020-08682-5u-TiO
2 CARTRIDGES, 3.0 I.D.	25020-08681-5u-TiO
2 CARTRIDGES, 4.0 I.D.	25020-08680-5u-TiO2
1 HOLDER, 2 CARTRIDGES, 1.0 I.D.	5020-08693-5u-TiO2
1 HOLDER, 2 CARTRIDGES, 2.1 I.D.	5020-08692-5u-TiO2
1 HOLDER, 2 CARTRIDGES, 3.0 I.D.	5020-08691-5u-TiO2
1 HOLDER, 2 CARTRIDGES, 4.0 I.D.	5020-08690-5u-TiO2

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

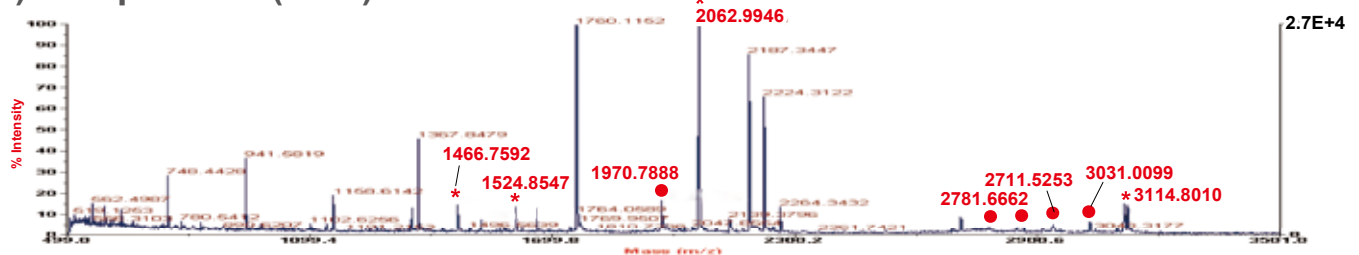
(a) Without treatment



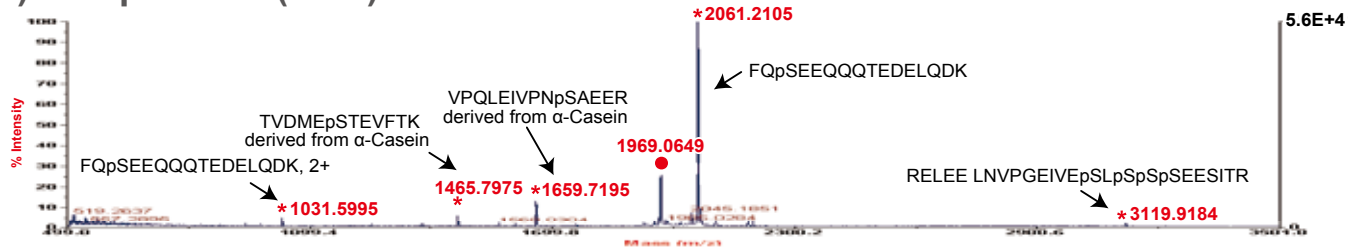
(b) Titansphere® Phos-TiO Kit



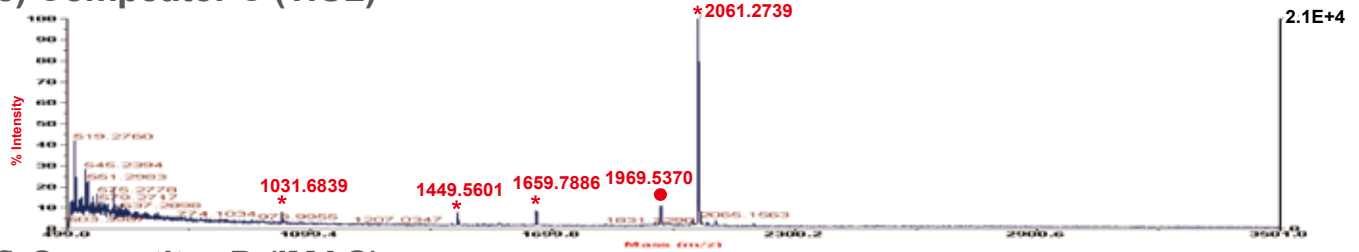
(c) Competitor A (TiO₂)



(d) Competitor B (TiO₂)



(e) Competitor C (TiO₂)



(f) Competitor D (IMAC)

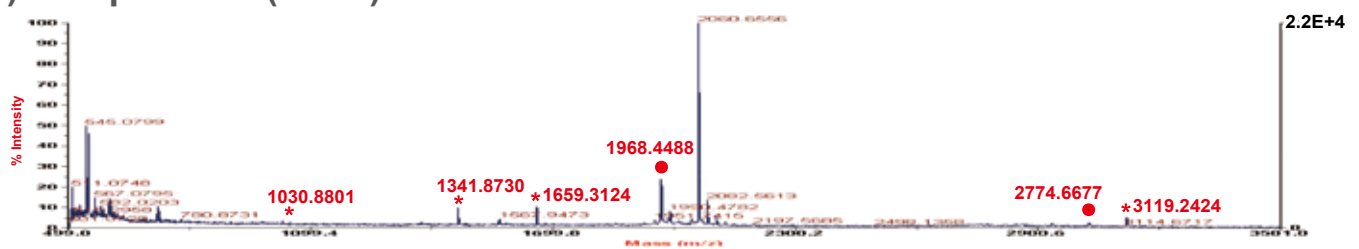


Figure 1. Comparison of Enrichment Efficiency of Tryptic Digest of 2.5µg β-casein by MALDI-TOF/MS

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-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).

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

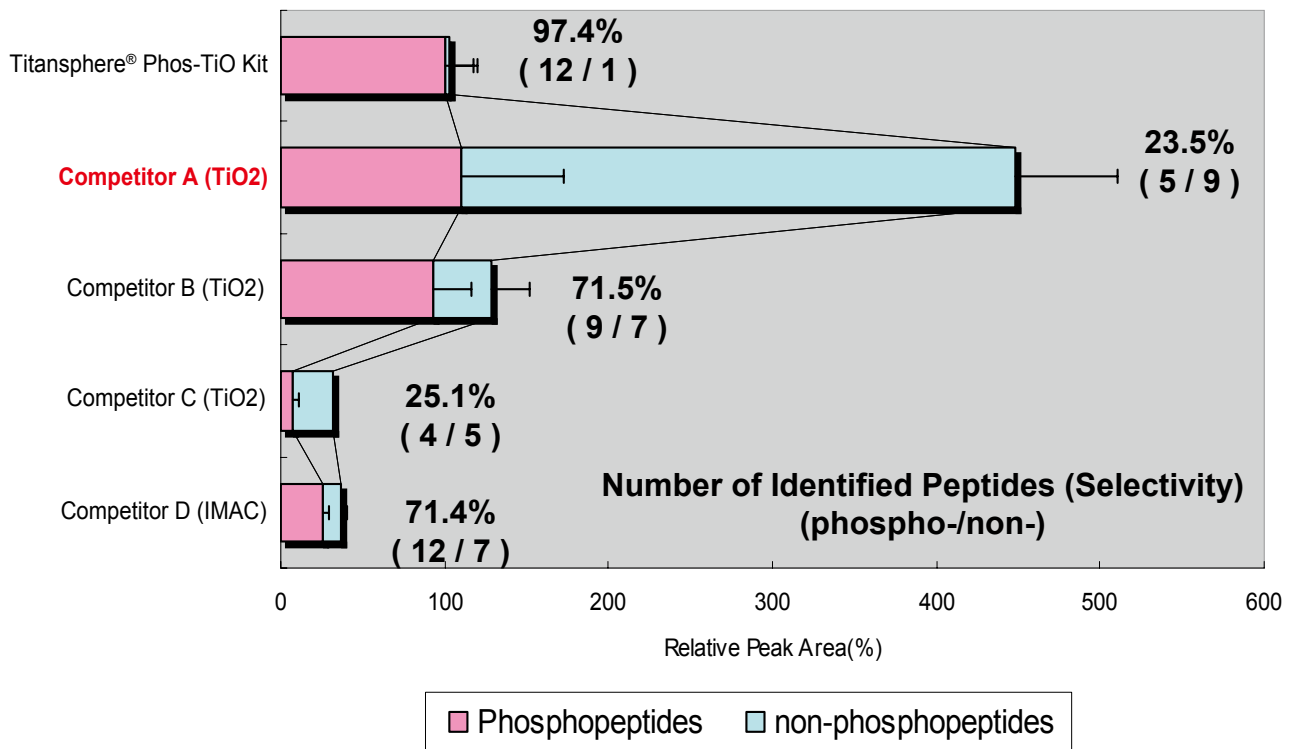


Figure 2. Comparison of Commercially Available Phosphopeptide Enrichment Kits by LC-MS.

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).

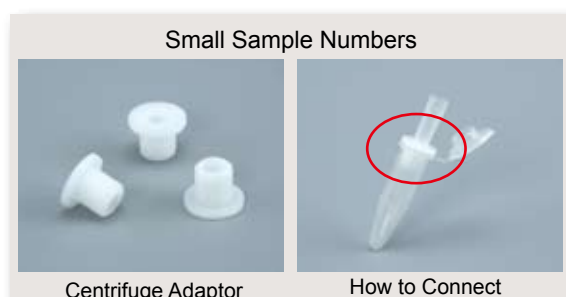
The % in the graph above shows the phosphopeptides peak area ratio within all the detected peptides peak area.

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.

Titansphere® Phos-TiO Kit

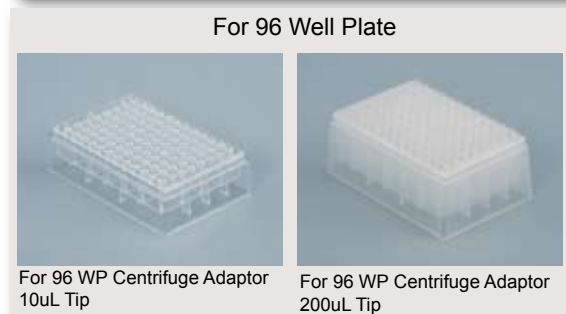
Product	Contents	Tip Volume	Particle Size/Packed Volume	Qty/Pack	Cat.No.
Titansphere® Phos-TiO Kit	Tip Column	10 µL	10 µm/1mg	24	5010-21300
	Solution A	—	—	2mL	
	Solution B	—	—	2mL	
	Tip Column	10 µL	10 µm/1mg	96	5010-21301
	Solution A	—	—	6mL	
	Solution B	—	—	6mL	
	Tip Column	200 µL	10 µm/3mg	24	5010-21305
	Solution A	—	—	2mL	
	Solution B	—	—	2mL	
	Tip Column	200 µL	10 µm/3mg	96	5010-21306
	Solution A	—	—	6mL	
	Solution B	—	—	6mL	

Titansphere® Phos-TiO Centrifuge Adaptor



Please purchase the following centrifuge adaptor once. These adaptors can be reused.

Product	Qty/Pack	Cat.No.
Centrifuge Adaptor (For 10uL and 200uL Tip)	24	5010-21514



Product	Qty/Pack	Cat.No.
96WP Centrifuge Adaptor for 10ul Tip	1	5010-21340
	2	5010-21342
96WP Centrifuge Adaptor for 200ul Tip	1	5010-21341
	2	5010-21343

Titansphere® TiO Beads

Product	Cat.No.
Titansphere®TiO 5 µm Beads 500mg	5020-75000
Titansphere®TiO 10 µm Beads 500mg	5020-75010
Empore™ DISK C8 Diameter47mm、Film Thickness 0.5mm、20(pcs)	5010-30002
Empore™ DISK C8 Diameter90mm Film Thickness 0.5mm、10(pcs)	5010-30003

※For 3um, please inquire.

MonoTip® Series (Pretreatment Pipetting Tip)

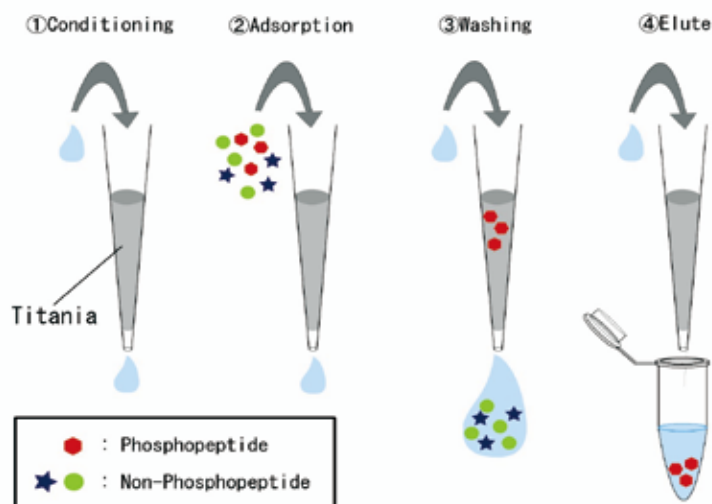
Product	Purpose	Volume	Qty/Pack	Cat.No.
MonoTip® Trypsin	Trypsin Digestion in Liquid Protein for 20 minutes	200 µ L	24	5010-21012
			96	5010-21010
MonoTip® C18	Desalting for Proteins and Peptides	200 µ L	24	5010-21002
			96	5010-21000
MonoTip® mini C18	Desalting for Proteins and Peptides	10 µ L	24	5010-21202
			96	5010-21200

Easy to Operate

The total operation is only 4 steps, and total operation time is only 40 minutes.

Centrifuge Operation

All the operations of "Titansphere® Phos-TiO Kit" use 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, 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 samples.



- The specification and the column type are subject to change without notice due to continual improvements.
- All brand names and product names are trademarks or registered trademarks of GL Sciences Inc.
- TM or ® are not described in this brochure.

GL Sciences Inc., is an ISO9001 Certified company.



22-1 Nishishinjuku 6-chome Shinjuku-ku, Tokyo, 163-1130 Japan
International Department TEL : +81-3(5323)6620 Fax : +81-3(5323)6621