Titansphere ® Phos-TiO Kit for export Instruction Manual

Thank you for purchasing the Titansphere® Phos-TiO kit.

The Titansphere® Phos-TiO Spin Tip is a sample preparation tip packed with synthesized spherically porous titanium dioxide (TiO_2), suited for purifying and enriching phosphopeptide. This spin tip is a customized product to separate and/or purify phosphopeptide from packing material, Titansphere TiO for high performance liquid chromatograpy (HPLC).

Our Titansphere® Phos-TiO Kit can be used for the purification and enrichment of phosphopeptide directly from cell culture medium, and/or samples roughly purified by immuno-precipitation, SDS-Page or the two-dimensional electrophoresis.

To maintain optimum performance, read the following instructions before use.

1. Product Description

Check for damage or missing parts.

Cat. No.	5010-21309	5010-21310	5010-21311	5010-21312
Titansphere® Phos-TiO Kit	24 pcs	96 pcs	24 pcs	96 pcs
Titansphere® TiO Bulk/Tip Volume	1 mg / 10 µL		3 mg / 200 µL	
Spin Tip	24 pcs	96 pcs	24 pcs	96 pcs
Waste Fluid Tube	24 pcs	96 pcs	24 pcs	96 pcs
Recovery Tube	24 pcs	96 pcs	24 pcs	96 pcs
Solution B	2 mL	6 mL	2 mL	6 mL
Instruction Manual	1 piece	1 piece	1 piece	1 piece

2. Handling

- Do not drop the tip or bump it on anything. Any strong impact may cause to splattering of the titanium gel.
- Do not autoclave.
- Please store the product in a low humidity environment such as desiccators, after the package of the tip is opened.

3. Notice

- All the experiments must be done with a centrifuge instrument. Use of syringes or other air forcing methods may result in varied efficiency.
- Surface-active agents and/or denatures such as SDS, urea, may cause the performance deterioration of the Spin tip. We recommend to optimizing an experimental condition by performing the preliminary exam, when you use such reagent.
- Use high-purity acetonitrile and trifluoroacetic acid, for MS.
- Titansphere® Phos-TiO Kit is a disposable product and not for reuse.
- Prepare the buffer just before performing the experiment. Do not use the buffer prepared ahead of time.

4. Before Experiments

1) Sample Preparation (Please prepare protein digestive fluid or peptides)

A) To detect the phosphopeptide in standard protein:

Prepare 15ulLof standard phosphopeptide digest protein at 0.5mg/mL

For example

α-Casein	Trypsin Digest at 0.5mg/mL	5µL	
Fetuin	Trypsin Digest at 0.5mg/mL	5µL	
Phosvitin	Trypsin Digest at 0.5mg/mL	5µL	Total 15µL

B) To detect the phosphopeptide in Hela cells:

50µL of Hela cell digest at 0.5mg/mL (total protein mass before digestion: 25ug)

2) Reagent Preparation

- High-purity acetonitrile
- High-purity trifluoroacetic acid (TFA)
- Ammonium Hydroxide solution
- Pyrrolidine

3) Reagent Preparation (When Treating 24 Samples)

- Prepare 2% TFA solution. Use 2%TFA solution and Acetonitrile to make Buffer A
- Use solution B provided within the product and Buffer A to make Buffer B

Buffer A	2% TFA solution.	1mL
	Acetonitrile	4mL
	Total	5mL（Use 3mL for making Buffer B）

Buffer B	Solution B	1mL
	Buffer A	3mL
	Total	4mL

- Prepare 5% Pyrrolidine solution.
- Prepare 5% Ammonium Hydroxide solution.

Note : Use the reagent made on the day of the experiment.

Prepare the reagent just before performing the experiment. Do not use it prepared ahead of time.

Solution B has a high viscosity. Aspirate slowly.

5. Protocol

- Set the centrifuging rate at 3,000 *xg*.
- Use digestive peptide after finishing the reduction and alkylation procedure.
- Make sure that the solution is completely eluted from the Spin Tip after each centrifuge operation.
- Regardless the Spin Tip volume (10µL or 200µL), use the same amount of Buffer except the Buffer B for adsorption (e.g.): Use 20µL of conditioning buffer for both types.

Spin Tip

– Connect a centrifugal adaptor to a Waste Fluid Tube, and insert the Spin Tip in to tube. (Fig.1)

Conditioning

– Add 20µL of Buffer A, then centrifuge. (3,000 *xg*. 2min, RT)

Equilibration

– Add 20µL of Buffer B then centrifuge. (3,000 *xg*. 2min, RT)
– Remove the 40µL of effluent from the Waste Fluid Tube

Adsorption

– Add 15~50µL of a sample and 100ul of Buffer B into the Spin Tip. (In the case of 10µL tip, add 15µL of the sample and 50µL of Buffer B into the Spin Tip.)
– To mix the sample with Buffer B, repeat pipetting three times within the Spin Tip. (Fig.2)
– Centrifuge. (1,000 *xg*. 10min, RT)
* High centrifugal speed may lead to determination in recovery rate.
– Put the sample in the tube back into the Spin Tip again and centrifuge. (1,000 *xg*. 10min, RT)
– Remove the sample and buffer B collected in the tube.

Rinsing

– Add 20µL of Buffer B and centrifuge. (3,000 *xg*. 2min, RT)
– Add 20µL of Buffer A and centrifuge. (3,000 *xg*. 2min, RT)
(Add of buffer A Repeat twice)

Elution

– Put the Spin Tip into the recovery tube
– Add 50µL of 5% ammonium hydroxide solution and centrifuge. (1,000 *xg*. 5min, RT)
– Add 50µL of 5% pyrrolidine solution and centrifuge. (1,000 *xg*. 5min, RT)

Phosphopeptide

RT = Room Temperature

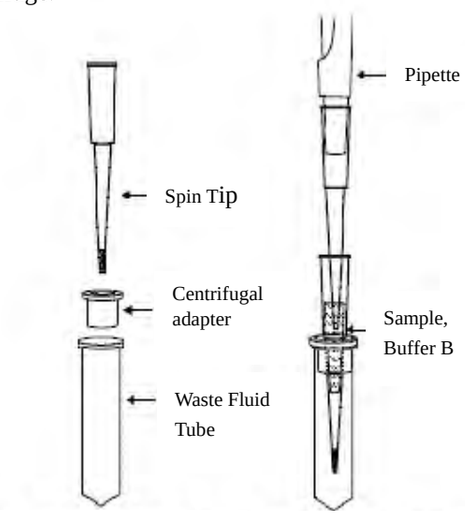

Fig.1 Setting of Spin Tip

Fig.2 Mixing of sample and buffer B

6. FAQ

Trouble	Possible Causes	Countermeasure
Cannot recover phosphopeptide	Low peptide concentration	Have peptide with more than 1 ug/mL concentration
Many interfering peaks besides Phosphopeptide are detected	Not enough rinsing	Increase the elution volume of Buffer B. Increase the acetonitrile concentration of Buffer B for rinsing.
	Not enough rinsing	Increase the number of rinsings with Buffer B.
	Inappropriate elution	Use 0.2M phosphate buffer (pH7.0).
Poor recovery Rate	Low adsorption	Increase the number of adsorption procedure.
	Liquid from the previous procedure remaining	Confirm the liquid is completely eluted after the centrifugal procedure.
	The tip end touches the waste fluid	The recovery rate and selectivity deteriorates if the tip end touches the waste fluid. Especially when the sample volume is large, make sure that the waste fluid is completely removed after adsorption.
Spectrum such as m/z = 486, 630, 774 appears	Not enough rinsing	Use Buffer A and rinse several times.

7. Storage

- Store the Titansphere® Phos-TiO Kit in a clean and dark place with a constant temperature.
- Keep the unpacked tips in a low humidity environment such as a desiccators.

Titansphere® Phos-TiO Kit is manufactured, inspected, packed and shipped under our strict standards of quality control. Please contact us if you find any problems with the performance of the product.

This product is only available for research purposes. We do no guarantee against using this product other than research purpose or other than usage described in this instruction.

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