

Titansphere[®] Phos-TiO Kit Operation Manual

Thank you for purchasing the Titansphere[®] Phos-TiO Kit.

The Titansphere[®] Phos-TiO Kit is an effective sample preparation tip to purify and enrich phosphopeptide packed with spherically synthesized titanium oxide crystal. This Titansphere[®] Phos-TiO Kit is a customized product of our Titansphere TiO bulk for phosphopeptide purification.

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

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

1. Unpacking

- Check for damage or missing parts.

Cat. No.	5010-21300	5010-21301	5010-21305	5010-21306
Titansphere [®] Phos-TiO Kit	24 pcs	96 pcs	24 pcs	96 pcs
Titansphere [®] TiO Bulk/Tip Volume	1mg / 10uL		3mg / 200uL	
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 A	2mL	6mL	2mL	6mL
Solution B	2mL	6mL	2mL	6mL
Operation Manual	1 pcs	1 pcs	1 pcs	1 pcs

2. Handling

- To prevent splattering, do not drop or knock the Tip or the Titansphere bulk.
- Titansphere[®] Phos-TiO Kit cannot be autoclaved.
- Keep the unpacked tips in a low humidity environment such as a desiccator.

3. Cautions

- All the experiments must be done with a centrifuge machine. Use of syringes or other air forcing methods may result in varied efficiency.
- Foreign substances in the sample can cause the digestive peptide density and solution volume of the initial sample to vary (due to the density or types of interfacial active agents or denaturants). Perform experiments in advance to determine the optimum conditions.
- Use highly pure acetonitrile for MS.
- Titansphere[®] Phos-TiO Kit is disposable and cannot be reused.
- Prepare the reagent on the same day as the experiment is to be performed. If performing the same experiment on the following day, re-prepare the reagent on the day.

4. Before Experiments

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

A. To detect the phosphopeptide in standard protein:

α -Casein	Trypsin Digest at 0.5mg/mL	5uL	
Fetuin	Trypsin Digest at 0.5mg/mL	5uL	
Phosvitin	Trypsin Digest at 0.5mg/mL	5uL	Total 15uL
Prepare 15ul of standard phosphopeptide digest protein at 0.5mg/mL			

B. To detect the phosphopeptide in HeLa cells:

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

② Reagent Preparation

What to Prepare:

- Acetonitrile (HPLC grade)
- Ammonium Water

③ Reagent Preparation (When Treating 24 Samples)

Use "Solution A and B" provided with in the product to make "Buffer A and B"

Buffer A	Solution A	1mL
	Acetonitrile	4mL
	Total	5mL (3mL is used to make Buffer B)

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

(Caution) 1. Use the reagent made on the day of the experiment.

If performing the same experiment on the following day, re-prepare the reagent on the day.

2. Solution B has a high viscosity. Aspirate slowly.

5. Protocol

- Use Buffer A and Buffer B made on the same day as the experiment.
- Prepare 0.5% NH₄OH solution.
- Set the centrifuge machine at 2,000 *xg*.
- Use digestive peptide after finishing the reduction and alkylation procedure.
- Make sure that the solution is completely eluted from the tip columns after each centrifuge operation.
- Regardless the tip column volume (10ul or 200ul), use the same amount of Buffer expect the Buffer B for absorption (EX: Use 20ul of conditioning Buffer for both 10ul and 200ul columns)

Tip Column

- Attach the centrifuge adaptor to the Waste Fluid Tube, and insert the tip column. (Fig. 1)

Conditioning

- Add 20ul of Buffer A, then centrifuge at 2,000 *xg*. 2mins, RT. (RT = Room Temperature)

Equilibrating

- Add 20ul of Buffer B then centrifuge at 2,000 *xg*. 2mins, RT.
- Remove the 40ul of waste fluid from the Waste Fluid Tube

Absorption

- Add 15~50uL of sample and 100ul of Buffer B to the tip column. (For the 10uL tip, add 15uL of sample and 50uL of Buffer B to the tip column.)
- To mix the sample and Buffer B, repeat drawing and ejecting a few times within the tip column. (Fig. 2)
- Centrifuge at 1,000 *xg*. 10mins, RT
- Add the sample in the Waste Fluid Tube to the tip column again then centrifuge at 1,000 *xg*. 10mins, RT
- Remove the sample in the Waste Fluid Tube and Absorbing Buffer B.

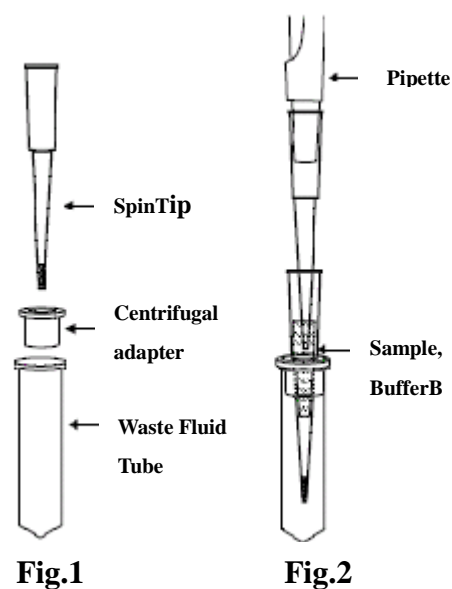
Rinsing

- Add 20uL of Buffer B then centrifuge at 2,000 *xg*. 2min, RT.
- Add 20uL of Buffer A then centrifuge at 2,000 *xg*. 2min, RT (Repeat twice)

Elution

- Put the tip column into the Recovery Tube
- Add 50uL of 0.5% NH₄OH then centrifuge at 1,000 *xg*. 5min, RT
- Add the solution in the Recovery Tube to the tip column again, then centrifuge (1,000 *xg*. 5min, RT)

Phosphopeptide



6. F A Q

Trouble	Predicted Causes	Countermeasure
Cannot recover phosphopeptide	Low peptide concentration	Have peptide with more than 1 ug/mL concentration
Many 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 phosphoric acid buffer (pH7.0) .
Poor Recovery Rate	Low absorption	Increase the number of absorption procedure.
	Liquid from the previous procedure remaining	Confirm the liquid is completely eluted after the centrifuge 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 absorption.
Spectrum such as m/z = 486,630, 774 appears	Not enough rinsing	Use Buffer A and rinse several times.

7. Storing

- Store the Titansphere® Phos-TiO Kit Spin Tip in a clean place with a constant temperature.
- **Keep Solution A in a refrigerator at 4°C.**
- **Keep the unpacked tips in a low humidity environment such as a desiccator**

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 manufactured for research purposes. We do no guarantee usage for purposes other than research or uses other than those described in the operations manual.



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