High-Select™ TiO_2 Phosphopeptide Enrichment Kit

Catalog Number A32993

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Product description

The Thermo Scientific™ High-Select™ TiO_2 Phosphopeptide Enrichment Kit enables efficient isolation of phosphorylated peptides from complex and fractionated protein digests for analysis by mass spectrometry (MS). Spherical porous titanium dioxide (TiO_2) resin spin tips combined with optimized buffers provide enhanced identification and enrichment of phosphopeptides with greater than 85% specificity. The optimized protocol and buffers result in a higher yield of phosphopeptides ready for direct MS analysis without the need for additional graphite or C18 clean up.

Mass spectrometry is a key tool for identifying sites of protein phosphorylation and quantifying phosphorylation changes. However, MS analysis of protein phosphorylation is challenging due to the low stoichiometry, high hydrophilicity, poor ionization and incomplete fragmentation of phosphopeptides. Because of the low relative abundance of phosphorylation modifications in complex protein samples, enrichment is essential for successful MS analysis of phosphopeptides. The improved High-Select™ TiO_2 Phosphopeptide Enrichment Kit is compatible with our lysis, reduction, alkylation, digestion and high pH reversed-phase peptide fractionation columns to provide a complete workflow for phosphopeptide enrichment.

Contents

Contents	Cat. No. A32992	Storage
High-Select™ TiO_2 Phosphopeptide Enrichment Kit	TiO_2 Spin Tips, 24 each	Store at 4°C.
	Centrifuge Column Adaptors, 24 each	
	Binding/Equilibration Buffer, 7 mL	
	Wash Buffer, 1.8 mL	
	Phosphopeptide Elution Buffer, 7 mL	

Additional information

- The Thermo Scientific™ Pierce™ Mass Spec Sample Prep Kit for Cultured Cells or urea-based lysis methods can be used to prepare peptide digest samples.[1]
- It is recommended to enrich phosphopeptides from lyophilized peptide samples free of detergents and salts. Ensure desalted peptide samples are completely dissolved in Binding/Equilibration Buffer for optimal results.
- Each spin tip can enrich phosphopeptides from 0.5mg to 3.0mg of a total protein digest starting sample. Phosphopeptide yields are typically ~1-3% of the starting sample tip load and can be determined using the Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay Kit (Product No. 23275).
- Using a pipette to draw and expel liquid through the spin tip is strongly discouraged and may result in poor, varied results. Centrifuge column adaptors are reusable.
- For optimal results, perform the procedure promptly and avoid excessive resin drying between steps.
- Equilibrate all solutions to room temperature prior to enrichment experiment. Securely tighten buffer bottle caps to prevent evaporation and store unused buffers and columns at 4°C.
- Humidity and exposure to direct sunlight lower performance of the TiO_2 tips. Seal aluminum pouch and keep in plastic bag after each use.

[1] Antharavally, *et al*. (2013) https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/protein-biology-application-notes/mass-spectrometry-sample-preparation-procedure-protein-samples.html

Perform phosphopeptide enrichment

Materials required but not provided

- Collection tubes: Low protein binding microcentrifuge tubes, 2 mL (Product No. 88379)
- Water, LC-MS Grade (Product No. 51140)
- 0.1% Formic acid, LC-MS Grade (Product No. 85170)
- pH paper
- *Optional:* Pierce™ Quantitative Colorimetric or Fluorometric Peptide Assay Kit (Product No. 23275 or 23290)

Suspend peptide sample

1. Completely suspend lyophilized peptide sample in 150 µL of Binding/Equilibration Buffer. Use vortex mixer with tube stand if necessary.
   Note: For optimal results, lyophilized peptide samples must be entirely dissolved in Binding /Equilibration Buffer.
2. *Optional*: Verify pH of resuspended sample is <3 using pH paper.

Prepare column

1. Place a Centrifuge Column Adaptor in a 2 mL collection tube and insert a TiO_2 Spin Tip into the adaptor.
2. Add 20 µL of Wash Buffer and centrifuge at 3000 × *g* for 2 minutes.
3. Add 20 µL of Binding/Equilibration Buffer and centrifuge at 3000 × *g* for 2 minutes.
4. Discard the flowthrough. Save the microcentrifuge tube for later "Wash column" step 1.

Bind phosphopeptide

1. Transfer the equilibrated TiO_2 Spin Tip and adaptor into a new 2 mL microcentrifuge tube.
2. Apply 150 µL of suspended peptide sample to the spin tip. Centrifuge at 1000 × *g* for 5 minutes.
3. Reapply sample in the microcentrifuge tube to the spin tip. Centrifuge at 1000 × *g* for 5 minutes. If desired, retain the flowthrough for analysis.

   Note: Applying sample twice to the spin tip results in an ~11% additional phosphopeptide yield.

Wash column

1. Transfer the TiO_2 Spin Tip and adaptor into the collection tube saved from "Prepare column" step 4.
2. Wash column by adding 20 µL of Binding/Equilibration Buffer. Centrifuge at 3000 × *g* for 2 minutes.
3. Wash column by adding 20 µL of Wash Buffer. Centrifuge at 3000 × *g* for 2 minutes.
4. Repeat steps 2 and 3.

   Note: Changing the order of wash column steps results in significantly higher nonspecific peptide binding.
5. Wash column by adding 20 µL of LC-MS grade water. Centrifuge at 3000 × *g* for 2 minutes.

Elute column

1. Remove excess liquid by blotting bottom of the spin tip on a clean lab tissue.
2. Place the spin tip and adaptor in a new collection tube and add 50 µL of Phosphopeptide Elution Buffer. Centrifuge at 1000 × *g* for 5 minutes. Repeat step once.
3. Dry the eluate immediately in a speed vacuum concentrator to remove Phosphopeptide Elution Buffer.

   Note: Eluates cannot be stored in Phosphopeptide Elution Buffer because high pH will lead to loss of phosphates on phosphopeptides.
4. Suspend the eluate with 50 µL 0.1% formic acid for peptide concentration measurements using the Pierce™ Quantitative Colorimetric Peptide Assay Kit or direct MS analysis.

   Note: For <1 mg starting peptide sample amounts, suspend dried elute using 25 µL of 0.1% formic acid.

Related products

Product	Cat. no.
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Trifluoroacetic Acid, Sequence Grade	28904
Water, LC-MS Grade	51140
Acetonitrile (ACN), LC-MS Grade	51101
High-Select™ Fe-NTA Phosphopeptide Enrichment Kit	A32992
Pierce™ Mass Spec Sample Prep Kit for Cultured Cells	84840
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	84868
Pierce™ Peptide Retention Time Calibration Mixture	88320
Pierce™ Detergent Removal Spin Columns, 0.5 mL	87777
Pierce™ C18 Tips, 10 µL bed	87782
Pierce™ C18 Tips, 100 µL bed	87784
Pierce™ C18 Spin Columns	87784
Pierce™ Graphite Spin Columns	88302
Pierce™ Peptide Retention Time Calibration Mixture	88320
Pierce™ HeLa Digest Protein Standard	88328
Pierce™ Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit	88811
Pierce™ Trypsin Protease, MS Grade	90057
Halt™ Phosphatase Inhibitor Cocktail	78427

Troubleshooting

Observation	Possible cause	Recommended action
No phosphopeptide recovered.	Phosphatase inhibitors were not used during protein extraction.	Add phosphatase inhibitors to protein extraction buffers.
	Phosphopeptide concentration is too low.	Increase amount of sample.
	Sample pH was >3.5 after suspension in Binding/Equilibration Buffer.	Desalt protein digest samples before suspending in Binding/Equilibration Buffer.
		Reduce pH to <3 by adding TFA.
	High level of interfering agents in the sample.	Modify protein sample preparation to remove detergents, EDTA, reducing agent and other interfering substances.
	Phosphopeptide lost during clean up.	Enriched phosphopeptide samples do not require additional C18 clean up.
		Avoid trap columns during LC-MS.
	LC-MS is not optimal for phosphopeptide analysis.	Check hydrophilic peptide retention on LC column using Pierce™ Peptide Retention Time Calibration Mixture (Product No. 88320).
		Optimize MS methods to avoid or trigger on phosphopeptide neutral loss.
Low phosphopeptide specificity.	Nonspecific peptides bound to plastics.	Use low protein binding microcentrifuge tubes (Product No. 88379).
	Washing steps performed in incorrect order.	Perform tip equilibration and washing steps in correct order.
Clogged spin tip.	Particulates in the sample due to incomplete dissolution of protein digest sample in Binding/Equilibration Buffer.	Use vortex mixer to completely dissolve the digest peptide sample.
		Centrifuge the sample before application to the spin tip.

Limited product warranty

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Revision history: Pub. No. MAN0016054

Revision	Date	Description
A.0	01 August 2016	New manual