High-Select™ Fe-NTA Phosphopeptide Enrichment Kit

Catalog Number A32992

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Product description

The Thermo Scientific™ High-Select™ Fe-NTA Phosphopeptide Enrichment Kit enables fast and efficient enrichment of phosphorylated peptides with greater than 90% specificity. The simplified procedure requires less than 45 minutes to enrich phosphopeptides from protein digests or peptide fractions for mass spectrometry (MS) analysis. Each spin column included in the kit contains a phosphopeptide-specific resin that offers excellent binding and recovery properties for enriching up to 150 µg of phosphopeptides per column.

Mass spectrometry is a key tool for identifying sites of protein phosphorylation and quantifying phosphorylation changes; however, MS analysis of protein phosphorylation is challenging because of 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™ Fe-NTA Phosphopeptide Enrichment Kit complements our lysis, reduction, alkylation, and digestion reagents, along with our C18, graphite spin, and high-pH reversed-phase peptide fractionation columns to provide a complete workflow for phosphopeptide enrichment.

Contents

Contents	Cat. No. A32992	Storage
High-Select™ Fe-NTA Phosphopeptide Enrichment Kit	Spin columns, 30 columns, 200 µL of resin slurry per column	Store at 4°C.
	White Luer plug (end cap), 30 plugs	
	Binding/Wash Buffer, 2 × 20 mL	
	Phosphopeptide Elution Buffer, 7 mL	

Additional information

- The Thermo Scientific™ Pierce™ Mass Spec Sample Prep Kit for Cultured Cells (Product No. 84840) or urea-based lysis methods can be used to prepare peptide digest samples. Refer to protein biology application note.[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/Wash Buffer for optimal results.
- Each column can enrich phosphopeptides from 0.5 mg to 5 mg of a total protein digest starting samples. Phosphopeptide yields are typically ~2-4% of the starting sample column load and can be determined using the Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay Kit (Product No. 23275). Avoid using solutions that contain >5 mM EDTA or reducing agents such as 2-mercaptoethanol, DTT or TCEP.
- 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.

[1]Antharavally, *et al*. (2013) A versatile mass spectrometry sample preparation procedure for complex protein samples. 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 200 µL of Binding/Wash Buffer. Use vortex mixer with tube stand if necessary.
   Note: For optimal results, lyophilized peptide sample must be completely dissolved in Binding/Wash Buffer for optimal results.
2. *Optional*: Verify pH of resuspended sample is <3 using pH paper.

Equilibrate column

1. Remove the bottom closure of the spin column and loosen the screw cap.
2. Place column in a 2 mL microcentrifuge collection tube. Centrifuge at 1000 × *g* for 30 seconds to remove storage buffer.
3. Remove the screw cap and save it for "Bind phosphopeptides" step 2.
4. Add 200 µL of Binding/Wash Buffer. Centrifuge at 1000 × *g* for 30 seconds and discard the flowthrough. Repeat this step once.
5. Cap the bottom of the column with a white Luer plug. Place the column with the plug into the empty microcentrifuge tube.

Bind phosphopeptides

1. Add 200 µL of the suspended peptide sample to the equilibrated spin column. Close the screw cap.
2. Mix the resin with the sample by holding the screw cap and very gently tapping the bottom plug for 10 seconds until the resin is in suspension. Do not vortex or invert the column to avoid splashing the resin inside the column wall.

   Note: Mixing samples by vortexing or inversion results in significantly higher nonspecific peptide binding.
3. Incubate for 30 minutes. Mix the resin gently every 10 minutes as described in step 2.
4. Carefully remove the bottom plug and the screw cap.

   Note: Do not squeeze the bottom of the plug during removal as liquid inside the plug can backflow into the spin column.
5. Place the column into the microcentrifuge tube. Centrifuge at 1000 × *g* for 30 seconds. Discard the flowthrough.

Wash column

1. Wash column by adding 200 µL of Binding/Wash Buffer. Centrifuge at 1000 × *g* for 30 seconds. Repeat this step two additional times for a total of 3 washes. Discard the flowthrough.
2. Wash column by adding 200 µL of LC-MS grade water. Centrifuge at 1000 × *g* for 30 seconds.

Elute column

1. Place column in a new microcentrifuge tube.
2. Add 100 µL of Elution Buffer to the column. Centrifuge at 1000 × *g* for 30 seconds. Repeat this step once.

   Note: Resin color may turn brown, but this is normal.
3. Dry the eluate immediately in a speed vacuum concentrator to remove Elution Buffer.

   Note: Eluates cannot be stored in Elution Buffer as high pH will lead to loss of phosphates on phosphopeptides.
4. Suspend the dried eluate with 70 µL of 0.1% formic acid for peptide concentration measurements using the Pierce™ Quantitative Colorimetric Peptide Assay Kit or direct LC-MS analysis.

   Note: For <1 mg of starting peptide sample amounts, suspend dried elute using 40 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™ TiO_2 Phosphopeptide Enrichment Kit	A32993
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™ 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.
	Low phosphopeptide concentration.	Increase amount of sample.
	Sample pH was >3.5 after suspension in Binding/Wash Buffer.	Desalt and lyophilize protein digest samples before suspending in Binding/Wash Buffer.
		Reduce pH < 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 was 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 phosphopeptide neutral loss.
Low phosphopeptide specificity.	Nonspecific peptides bound to plastics.	Avoid excessive mixing of sample with resin or mix the resin with sample only once at the beginning of incubation.
		Use low protein binding microcentrifuge tubes.

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Revision	Date	Description
A.0	01 August 2016	New manual

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