High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit

Catalog Numbers A52283 and A52284

Doc. Part No. 2162753 Pub. No. MAN0025826 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ High-Select™ Fe-NTA Magnetic 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. The kit contains a phosphopeptide-specific resin that offers excellent binding and recovery properties for enriching 250-1000 µg of phosphopeptides per reaction.

Because of the relatively low abundance of phosphorylation modifications in complex protein samples, enrichment is essential for successful MS analysis of phosphopeptides. The High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit can be used to process samples manually or through automation using an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor.

Contents and storage

Product	Contents	Cat. No.	Storage
High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit	Fe-NTA Magnetic Agarose, 0.5 mL	A52283	4°C
	Binding/Wash Buffer, 20 mL		
	Phosphopeptide Elution Buffer, 1.8 mL		
High-Select™ Fe-NTA Magnetic Agarose	Fe-NTA Magnetic Agarose, 5 mL	A52284	

Additional information

- Do not dry or freeze the beads. Handling the beads in this way will cause the beads to aggregate and lose binding capacity.
- We recommend 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.
- Phosphopeptide yields are typically 2–4% of the starting sample column. Peptide concentration can be determined using the Pierce™ Quantitative Colorimetric Peptide Assay Kit (Cat. No. 23275). Avoid using solutions that contain >5 mM EDTA or reducing agents such as 2-mercaptoethanol, DTT, or TCEP.

Manual enrichment of phosphate-modified peptides

Required materials not supplied

- 1.5 mL low protein binding microcentrifuge tubes (Cat. No. 90410)
- Magnetic stand (e.g., DynaMag™-2 Magnet, Cat. No. 12321D)

Manually enrich phosphate-modified peptides

This protocol is designed to enrich phosphorylated peptides using the High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit (Cat. No. A52283). Lyophilize clean peptide samples using a speedvac before enrichment. Other sample types may require more resin for complete enrichment.

1. Dissolve 500 µg of clean peptide using 250 µL of Binding/Wash Buffer to a final concentration of 2 mg/mL in a low protein binding tube.
2. Transfer 10 µL of the magnetic Fe-NTA bead slurry using a cut or wide bore pipet tip to a new microcentrifuge tube and remove the storage buffer using a magnetic stand.
3. Wash the resin by adding 20 µL of Binding/Wash Buffer, then vortex briefly and remove the buffer using a magnetic stand.
4. Repeat the wash step once for a total of 2 washes.
5. Add 10 µL of Binding/Wash Buffer to the resin and transfer the bead slurry to the peptide solution (1:50, µL bead slurry:µg sample).
6. Incubate at room temperature for 30 minutes with end-over-end mixing.
7. Collect the beads using a magnetic stand and remove the unbound peptide solution.
8. Wash the resin by adding 20 µL of Binding/Wash Buffer, then vortex briefly and remove the buffer using a magnetic stand.
9. Repeat the wash step twice for a total of 3 washes.
10. Wash the resin by adding 20 µL of MS-grade water, then vortex briefly and remove the water using a magnetic stand.
11. Add 20 µL of Phosphopeptide Elution Buffer, then vortex briefly and incubate for 1 minute at room temperature.
12. Repeat the elution step once for a total of 2 elutions.

13. Combine the eluted phosphopeptide samples, transfer to a low protein binding tube, and speedvac dry before LC-MS analysis.

    Note: We recommend centrifugation of eluted peptide samples at 10,000 × *g* for 1 minute before transferring supernatant to avoid bead carryover before drying. We also recommend using C18 tips or trap columns to ensure resin particles do not interfere with LC-MS analysis.
14. Resuspend dried phosphopeptide sample using 0.1% FA for LC-MS analysis.

Automated enrichment of phosphate-modified peptides

Required materials not supplied

- KingFisher™ Flex Magnetic Particle Processor with 96 deep-well magnet (Cat. No. 5400630) or KingFisher™ Duo Prime Magnetic Particle Processor (Cat. No. 5400110)
- KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene (100–1000 µL; Cat. No. 95040450)
- 96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle Processor (Cat. No. 97002534)
- Binding/Wash Buffer: 80% acetonitrile, 0.1% TFA; 100 mL
- Phosphopeptide Elution Buffer: 5% ammonium hydroxide; 10 mL

Automatically enrich phosphate-modified peptides

Note: The following protocol is designed for use with the KingFisher™ Flex Magnetic Particle Processor. The protocol can be modified according to customer needs using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Download the appropriate BindIt™ Software protocol from the product page (Cat. No. 78605, 78606) from the Thermo Fisher Scientific website into the BindIt™ Software on an external computer.
2. Transfer the protocol to the KingFisher™ Flex instrument from an external computer. See the BindIt™ Software user manual for detailed instructions on importing protocols.
3. Set up plates according to Table 2.

   Table 1 Plate set-up.

Plate Number	Plate Name	Content	Volume
1	Tip plate	—	—
2	Beads	10 µL magnetic Fe-NTA agarose in 90 µL Binding/Wash Buffer	100 µL
3	Bead Wash	Binding/Wash Buffer	100 µL
4	Sample plate	Peptides in Binding/Wash Buffer	500 µL
5	Wash 1	Binding/Wash Buffer	100 µL
6	Wash 2	Binding/Wash Buffer	100 µL
7	Wash 3	Binding/Wash Buffer	100 µL
8	Wash 4	LC-MS-grade water	100 µL
9	Elution 1	Phosphopeptide Elution Buffer	50 µL
10	Elution 2	Phosphopeptide Elution Buffer	50 µL

   - If fewer than 96 wells are used, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
   - To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the beads to the plate.
   - Combine the Tip Comb with a 96 Deep-Well Plate. See the instrument user manual for detailed instructions.
4. Execute the phosphopeptide purification protocol on the KingFisher™ Flex instrument
5. Select the protocol using the arrow keys on the instrument keypad and press **Start**. See the KingFisher™ Flex Magnetic Particle Processor instrument user manual for detailed information.
6. Slide open the door of the instrument protective cover. Load plates into the instrument according to the protocol requests, placing each plate in the same orientation. Confirm each action by pressing **Start**.
7. After sample processing, remove the plates as instructed by the instrument display. Press **Start** after each plate. Stop after removing all the plates.
8. Combine the eluted peptides into a low protein binding tube/plate and speedvac dry.
9. Clean up eluted peptides using C18 tips, peptide desalting columns or EasyPep™ peptide clean-up columns.

   Note: Phosphopeptide clean up is required to remove leachables introduced from KingFisher™ 96 Deep-Well Plates before LC-MS analysis.
10. Resuspend dried phosphopeptide sample using 0.1% FA for LC-MS analysis.

Related products

Product	Cat. no.
High-Select™ TiO_2 Phosphopeptide Enrichment Kit	A32993
High-Select™ Fe-NTA Phosphopeptide Enrichment Kit	A32992
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Pierce™ Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit	88811
Pierce™ C18 Spin Tips	84850
Pierce™ Peptide Desalting Spin Columns	89851
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	84868
EasyPep™ Mini MS Sample Prep Kit	A40006
Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT), Single Use	A52285
DSPP (Disuccinimidyl Phenyl Phosphonic Acid, PhoX)	A52286
TBDSPP (tert-Butyl Disuccinimidyl Phenyl Phosphonate, tBu-PhoX)	A52287

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 the 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 agents, and other interfering substances.
	LC-MS was not optimal for phosphopeptide analysis.	Check the hydrophilic peptide retention on the LC column using Pierce™ Peptide Retention Time Calibration Mixture (Cat. No. 88320).
		Optimize MS methods or trigger phosphopeptide neutral loss.
Low phosphopeptide specificity	Nonspecific peptides bound to plastics.	Use low protein binding microcentrifuge tubes.
	Poor resin washing.	Increase the number of washes.
	Incubation time was too long in the elution buffer.	Reduce the time in the elution buffer to ≤1 minute.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	18 November 2021	New manual for the High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit.