High Select Fe-NTA magnetic beads for phosphopeptide enrichment

Erum Raja, Leigh Foster, Amarjeet Flora, Bhavin Patel, Ryan Bomgarden
Thermo Fisher Scientific, Rockford IL USA

Abstract

Purpose: Develop a new Thermo Scientific™ High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit for phosphopeptide enrichment from complex biological samples.

Methods: High Select Fe-NTA magnetic kit contains pre-formulated buffers, proprietary magnetic beads and an optimized protocol to successfully enrich different biological samples for phosphopeptide enrichment.

Results: High Select Fe-NTA magnetic kit can successfully enrich samples with a phosphospecificity of 90% or greater and with low CVs (<5%) between replicates.

Introduction

Mass spectrometry (MS) is a powerful tool for identifying sites of protein phosphorylation and quantifying phosphorylation changes. However, due to low abundance of phosphorylation modifications in a complex sample, enrichment is essential for successful MS analysis of phosphopeptides. Spin column-based Fe-NTA offers a convenient method to enrich phosphopeptides from a sample but remains a bottleneck for methods that require higher throughput. Magnetic Fe-NTA beads can seamlessly substitute Fe-NTA spin columns while offering great customer experience, scalability, and an automation-friendly workflow. Here, we have developed a new Magnetic Fe-NTA kit that can successfully enrich complex samples with a phosphospecificity of 90% or greater.

We have compared our beads to existing resins and magnetic beads and consistently seen high performance without any loss or bias towards specific phosphopeptide groups. We have also assessed the workflow on the Thermo Scientific™ KingFisher™ platform and demonstrated increased compatibility versus traditional phosphoenrichment formulations, which ensures reproducibility and eliminates the hands-on challenges of handling many samples. In addition, our newly developed High Select Fe-NTA magnetic beads are compatible with common solvents and over a wide pH range (e.g., pH 2-13). The excellent solvent and pH compatibility properties minimize the bead aggregation, adsorption and leaching on plastic surfaces, and degradation in high pH conditions during sample preparation.

Materials and methods

Sample Preparation

HeLa cells were cultured in DMEM media supplemented with 10% FBS and 1% Pen/Strep. Cells were treated with Nocodazole 0.1µg/ml for 24 hours. HeLa cells were harvested and lysed prior to protein concentration determination by Thermo Scientific™ Pierce™ BCA Protein Assay kit. Lysates were reduced, alkylated, and digested overnight prior to tC18 clean up. Peptide concentration was determined using Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay kit.

Phosphoenrichment

High Select Magnetic Fe-NTA beads were incubated in a blocking buffer for 5 minutes. The beads were combined with HeLa protein digest at a ratio of 1:50 (w:w) and incubated for 30 minutes. The beads are sequentially washed with wash buffers A and B to remove non-specifically bound peptides and other impurities. Phosphorylated peptides were eluted using an elution buffer and dried using a speedvac before mass spectrometry analysis. Automated enrichment of phosphopeptides was also performed using the Thermo Scientific™ KingFisher™ Apex Purification System.

LC-MS Analysis

Samples are analyzed by injection onto a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano system with separation over 105-minute gradient from 2-32% B using a Thermo Scientific™ EASY-Spray™ C18 column (PN#E903) coupled to a Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap MS or Thermo Scientific™ Orbitrap Exploris™ 480 MS. Thermo Scientific™ Proteome Discoverer™ 3.1 software was used for data analysis.

Figure 1. Schematic of phosphoenrichment workflow using High Select Fe-NTA magnetic beads

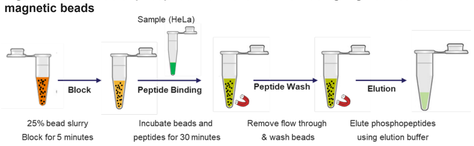
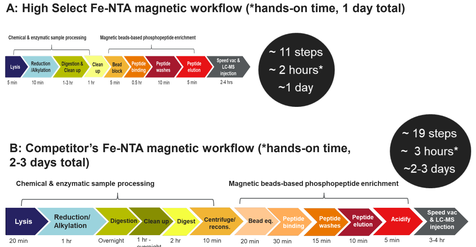


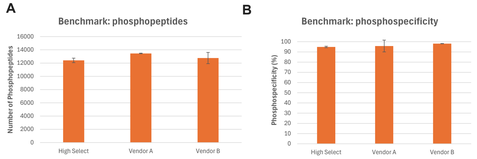
Figure 2: Comparison of High Select Fe-NTA magnetic workflow with competitor's workflow. Our workflow (A) is optimized to yield a high number of phosphopeptides in less than 11 steps and 2 hours of hands-on time which is a significant improvement over traditional workflow by competitors (B) which can take 19 steps and up to 3 days.



Results

Benchmark of High Select Fe-NTA magnetic resin

Figure 3: Benchmark of High Select Fe-NTA magnetic bead with other competitors. Our newly developed High Select Fe-NTA magnetic bead was benchmarked against two competitors alongside magnetic agarose (MagAg). The number of identified phosphopeptides (A) and phosphospecificity (B) was comparable among different beads. (C) High Select resulted in higher binding capacity of ~19ug when compared to a competitor's bead. (D) MS chromatogram is comparable among different Fe-NTA magnetic beads.



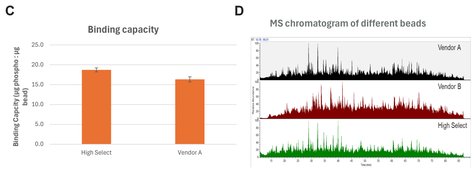


Figure 4. Lot to lot reproducibility. Nine different lots of High Select Fe-NTA magnetic beads were prepared and used to enrich HeLa digest samples. The results showed excellent performance with identification of ~12,000 phosphopeptides and ≥ 90% phosphospecificity among different lots.

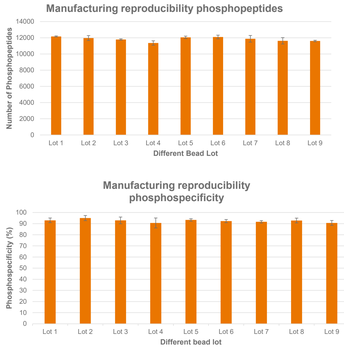
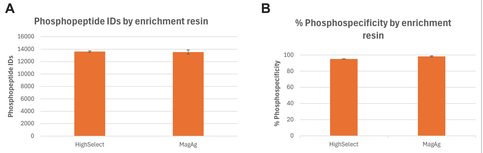
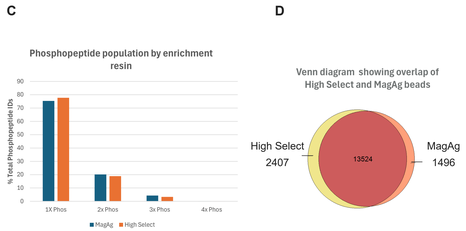


Figure 5. Performance by enrichment resin. High Select Fe-NTA magnetic resin was compared to a Fe-NTA magnetic agarose (MagAg) support. Both resins were comparable in terms of phosphopeptide IDs (A) and phosphospecificity (B). A similar phosphopeptide population was observed between the two resins (C). High overlap of quantifiable phosphopeptides (>85%) between the two resins (D).

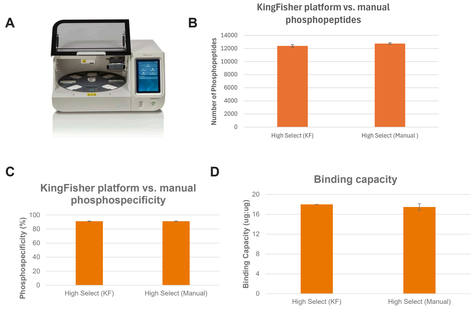




Applications

KingFisher automation workflow

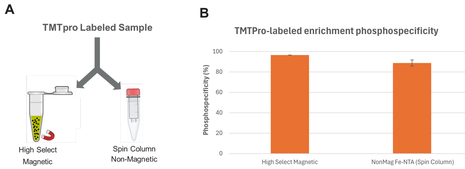
Figure 6. Automation workflow on KingFisher platform. A set of samples were analyzed on KingFisher platform (A) to show the utility of High Select Fe-NTA magnetic beads on an automation platform. Number of phosphopeptides (B) and % phosphospecificity (C) were almost identical when processed on KingFisher platform vs. manually. (D) Both manual and automated workflows had high binding capacities of >17µg:µg of bead.



TMT-labeled workflow

We evaluated High Select Fe-NTA magnetic beads with Thermo Scientific™ TMT™ workflow as TMT-labeled peptides are often an integral part of phosphopeptide enrichment. Following the protocol, digested sample was labeled with Thermo Scientific™ TMTpro™ labels, cleaned up using a tC18 column and washed with 0.1%TFA, 5% methanol. Phosphopeptide enrichment and LC-MS analysis was performed as described in the methods.

Figure 7. TMTpro phosphopeptide enrichment. TMTpro-labeled phosphopeptides (A) were enriched using High Select Fe-NTA magnetic beads and non-magnetic High Select Fe-NTA Phosphopeptide Enrichment kit (spin columns). High Select Fe-NTA magnetic beads outperformed traditional agarose resins, resulting in >95% phosphospecificity (B).



Conclusions

We have developed a new High Select Fe-NTA magnetic kit containing pre-formulated buffers, proprietary magnetic beads and an optimized protocol to successfully enrich a variety of different biological samples for phosphopeptide enrichment.

- Our High Select Fe-NTA magnetic beads can successfully enrich samples with high number of phosphopeptide IDs (>12,000) and >90% phosphospecificity.
- Our workflow is optimized to yield a high number of phosphopeptides in less than 11 steps and 2 hours of hands-on time which is a significant improvement over traditional workflow by competitors which can take 19 steps and up to 3 days.
- We have evaluated our beads against existing resins and magnetic beads and consistently observed superior performance without any loss or bias towards particular phosphopeptide groups.
- Additionally, we tested the workflow on the KingFisher platform and found it to be more compatible than traditional phosphoenrichment formulations, ensuring reproducibility and reducing the hands-on challenges associated with managing multiple samples.
- Our newly developed magnetic beads has excellent solvent and plastics compatibility and work over a wide pH range without any leachable or contaminants.
- Lastly, our High Select Fe-NTA magnetic beads are compatible with TMT workflow.

References

1. Paulo, Joao A., Liu et al. Fe3+-NTA Magnetic Beads as an Alternative to Spin Column-based Phosphopeptide Enrichment. ***Jour. Of Proteomics*** 260 (2022) 104561.
2. Kimura, Yayoi et al. Evaluation of Four Phosphopeptide Enrichment Strategies for MS based Proteomic Analysis. ***Proteomics*** 2022, 22:2100216

Trademarks/licensing

For Research Use Only. Not for use in diagnostic procedures.