A Novel Proteomics Magnetic Clean-up Bead for Automated Mass Spectrometry Sample Preparation

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Abstract

Here we present the utility of our newly developed Thermo Scientific™ Pierce™ Proteomics Magnetic Clean-Up Beads in preparing samples in different detergent lysis buffers for mass spectrometry (MS) analysis. The magnetic aspect of the bead allows for automated sample processing using the Thermo Scientific™ KingFisher™ instruments.

Here, we first used BSA (bovine serum albumin) with different detergents for sample preparation using our beads. Our MS results showed efficient removal of non-MS compatible detergents and good digestion efficiency of BSA protein. Next, we further verified the optimized magnetic bead protocol using mammalian cells and tissue samples to demonstrate compatibility with various detergents and lysis solutions (commercially available Thermo Scientific™ lysis reagents and kits).

The MS results showed no carry over of any detergents and comparable protein and peptide identifications across different samples, buffers, and fractions. The Proteomics Magnetic Clean-Up Beads was applied to the quantitative analysis of human lung tumor tissues and normal tissues, demonstrating excellent quantitative reproducibility with known specific protein biomarkers validation.

Introduction

Magnetic beads offer rapid and efficient processing of multiple samples simultaneously, helping save time and resources. In addition, magnetic bead-based workflows are amenable to automation, helping reduce the risk of human error and enabling consistent results for high-throughput proteomics sample preparation and analysis. However, some challenges exist which can complicate its adoption into the field. These challenges include the variety magnetic bead options, potential issues with reproducibility, and the need for standardized procedures.

Herein, we developed our Proteomics Magnetic Clean-up Beads with a workflow to enable fast, efficient, and reproducible removal of a wide variety of detergents, salts and other MS-incompatible contaminants using a variety of commercially available lysis reagents (Figure 1, Table 1).

Materials and methods

Proteomics Magnetic Clean-up Bead workflow
Samples were reduced and alkylated at 95˚ C for 15 minutes. Magnetic beads were washed and added to the sample with a binding solution (99.5% IPA, 0.5% TFA). Samples were incubated at room temperature with vigorous mixing for 45 minutes. The beads were then collected, supernatant was removed, and washed with 100% acetonitrile followed by 70% ethanol, 30% water. After washing, a digestion mixture of trypsin/Lys-C was added to the beads and incubated with vigorous mixing for 3 hours at 37˚ C. Supernatant was collected, dried in a vacuum concentrator, and resuspended in 0.5% formic acid. This same workflow was used on the Thermo Scientific™ KingFisher™ Apex Purification System for automation.

BSA Sample Preparation
50 µg of BSA stock was dried in a vacuum concentrator overnight, then resuspended in the different lysis reagents. These samples were then processed using the Proteomics Magnetic Clean-Up Beads workflow as shown in Figure 2.

Sample Lysis
HEK293 cells were processed per the manufacturer's instructions for the different buffers listed in Table 1. Mouse kidney was used for T-PER and human lung tissue was used in IP Lysis Buffer. 50 µg of total protein from each sample was processed as above using the Proteomics Magnetic Clean-Up Beads (Figure 2).

LC-MS/MS Analysis
A Thermo Scientific™ Vanquish™ Neo UPLC system equipped with a Thermo Scientific™ EASY-Spray™ PepMap™ Neo column (75 µm x 50 cm) coupled to a Thermo Scientifc™ Orbitrap Exploris™ 480 mass spectrometer using a 65 or 160 min gradient time, or coupled to an Ionopticks Aurora Frontier™ TS 60 cm nanoflow UHPLC column Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer equipped with FAIMS Pro Duo Interface using a 60 min gradient time, was used for analysis of different protein digest samples from cells, subcellular fraction or tissues. Raw files were analyzed using Spectronaut™, DIA-NN, and Thermo Scientific™ Proteome Discoverer™ 3.1 software with CHIMERYS™ intelligent search algorithm.

Results

Comparison of MS sample prep methods.

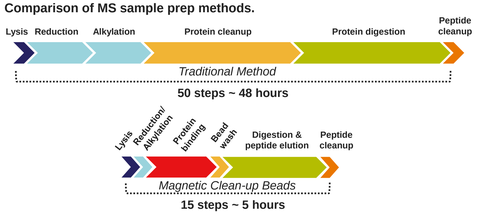

Figure 1. Comparison of traditional vs magnetic clean-up beads (PAC-SP3) MS sample prep methods. Top bar depicts the traditional MS sample preparation for incompatible detergents. Bottom bar depicts the Proteomics Magnetic Clean-up Bead workflow. Total time is reduced from 48 hours down to 5 hours.

Proteomics Magnetic Clean-up Bead workflow

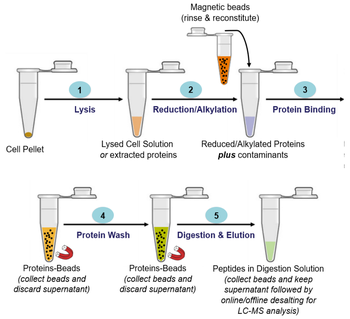

Figure 2. Proteomics Magnetic Clean-up Beads are used after lysis and reduction/alkylation steps for protein aggregation and capture (PAC) step.

Table 1. Thermo Fisher Scientific lysis reagents used in this study.

Lysis Reagent	Description
Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Reagents	Obtain nuclear and cytoplasmic fractions of soluble proteins from the same sample
Thermo Scientific™ Pierce™ GPCR Extraction and Stabilization Reagent	Optimized to extract and stabilize GPCRs for downstream activity assays
Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit	Obtain membrane and cytoplasmic fractions from the same sample
Thermo Scientific™ T-PER™ Tissue Protein Extraction Reagent	Optimized for mild extraction of total protein from tissue samples
Thermo Scientific™ Pierce™ IP Lysis Buffer	Optimized for IP and pull-down assays; maintains protein complexes
Thermo Scientific™ EasyPep™ MS Sample Prep Kit lysis buffer	General lysis buffer optimized for MS sample prep

Experimental workflow for evaluation of different lysis buffers/kits

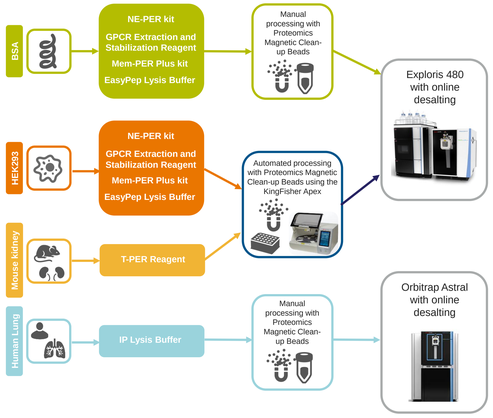

Figure 3. Different lysis buffers/kits and manual/automated proteomics magnetic clean-up beads methods were used to process cell lines/tissue samples.

MS results of the Proteomics Magnetic Clean-up Bead workflow with BSA

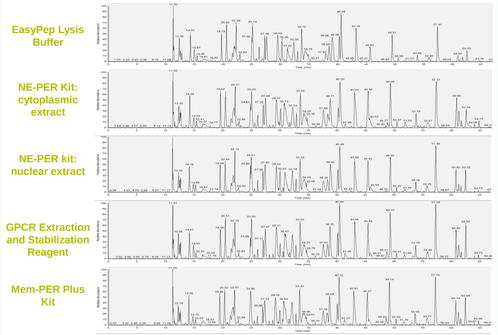

Figure 4. The resulting BSA total ion chromatograms (TICs) show very similar peptides separation and peak resolutions, including the EasyPep Lysis Buffer that is referenced as a positive control. No visible detergent contaminant peaks are seen throughout the gradient indicates efficient removal of detergents using proteomics magnetic clean-up beads.

	Sequence Coverage	Unique Peptides	0% Missed Cleavages
EasyPep lysis buffer	88%	102	58.6%
NE-PER kit, cytoplasmic extraction buffer	92%	114	63.6%
NE-PER kit, nuclear extraction buffer	86%	105	60.4%
GPCR Extraction & Stabilization Reagent	89%	100	57.8%
MemPER Plus, membrane extraction buffer	87%	102	52.9%

Table 2. BSA LC-MS analysis. Sequence coverage, unique peptides, and 0% missed cleavages are shown for the 5 different BSA samples processed using the Proteomics Magnetic Clean-up Beads. >85% sequence coverage, >100 unique peptides, and >50% zero missed cleavages for BSA was obtained using all 5 different buffers. These LC-MS results are very similar regardless of the starting lysis reagent.

MS results of the Proteomics Magnetic Clean-up Bead workflow with different sample types and lysis reagents

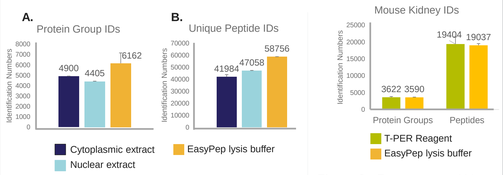

Figure 5. HEK293 cells were processed according to the manufacturer instructions, ending with two fractions from the same sample using the NE-PER kit. 50 µg of total protein was used for processing. Resulting protein group identifications (A) and unique peptide IDs (B) are shown.

Figure 6. Frozen mouse kidney was processed according to the manufacturer instructions. 50 µg of total protein was used for processing with the Proteomics Magnetic Clean-up Beads workflow. Resulting protein group identifications and unique peptide IDs are shown.

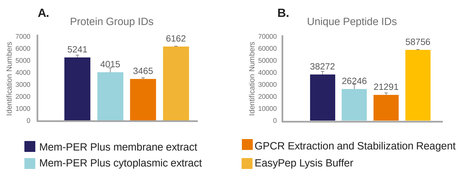

Figure 7. HEK293 cells were processed according to the manufacturer instructions for, ending with two fractions from the same sample using the Mem-PER Plus kit. 50 µg of total protein was used for processing. Resulting protein group identifications (A) and unique peptide IDs (B) are shown.

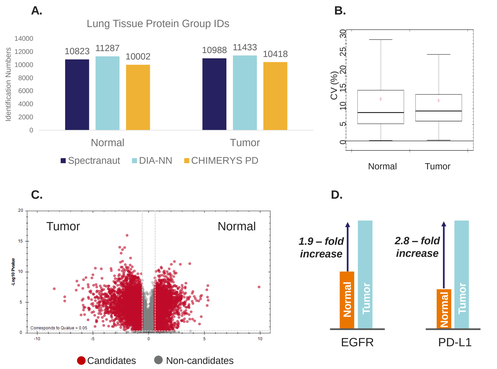

Figure 8. Human lung tissue, normal and tumor, was lysed using IP Lysis Buffer. 50 µg of total protein was used for processing. A. Protein group IDs are shown when the data is analyzed by three different software. B. These runs exhibited good quantitation precision with a CV of ~8% and 85% of the identified protein groups have CVs less than 20%. C. The volcano plot displays significant changes in abundance between the tumor and normal tissues. Red indicates potential for further investigation. D. Two of the significant changes are EGFR with a 1.9-fold increase in the tumor sample and PDL-1 with a 2.8-fold increase in the tumor sample. These two protein biomarkers are known to be highly expressed in in lung tumors compared to normal. [1,2]

Conclusions

- The Proteomics Magnetic Clean-up Bead workflow enables rapid and efficient processing of mammalian cells, tissues, and other samples for MS-based proteomics.
- The Proteomics Magnetic Clean-up Beads are compatible with automation platforms such as KingFisher for high-throughput proteomic sample preparation.
- Our Proteomics Magnetic Clean-up Beads help maximize laboratory productivity while significantly improving the speed and reproducibility of high-quality proteomics sample preparation.
- The Proteomics Magnetic Clean-up Beads are compatible with a variety of lysis reagent showing efficient detergent removal.
- Our Proteomics Magnetic Clean-up Beads enable quantitative analysis of human lung tissue to show biological significance.

References

1. Mitsudomi, T., & Yatabe, Y. (2010). Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. The FEBS Journal, 277(2), 301–308.
2. Garon, E. B., Rizvi, N. A., Hui, R., et al. (2015). Pembrolizumab for the treatment of non-small-cell lung cancer. The New England Journal of Medicine, 372(21), 2018–2028.

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