Optimized enrichment and analysis of MHC-I peptides for comprehensive immunopeptidome profiling

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Abstract

This study presents an optimized sample preparation solution to reproducibly enrich immunopeptides from cell culture with compatibility for automation using a magnetic bead-based approach. Enrichment of major histocompatibility class I (MHC-I) peptides was achieved by direct coupling of W6/32 antibody to magnetic beads, followed by immunoprecipitation from cell lysate, C18 clean-up including an internal standard for quality control, and LC-MS/MS analysis with de novo peptide sequencing. When combined with new Thermo Fisher Scientific mass spectrometers, this workflow enables reproducible sample preparation with in-depth coverage with accurate and precise quantitation of the immunopeptidome at scale.

Introduction

The immunopeptidome is the key to the immune system's ability to discriminate self from non-self, which creates the basis for immunotherapy. Identifying and quantifying the immunopeptidome is crucial for understanding the immune system and advancing precision medicine and cancer vaccines. Currently, there is no optimized sample prep solution to reproducibly enrich immunopeptides.

Here we present an immunopeptidomics sample prep workflow using Pierce™ Protein A/G Magnetic Agarose beads coupled to W6/32 antibody and modifications to a published protocol[1] to achieve reproducible enrichment of MHC-I peptides from cell lysate. A robust C18 tip peptide clean-up method, including a quality control internal standard, enables high peptide recovery and reproducibility. The use of magnetic beads allows for automated sample processing using the Thermo Scientific™ KingFisher™ instruments.

Materials and methods

Cell culture and lysis

HCT116 cells (ATCC) were maintained with and without treatment of 10ng/mL interferon gamma (IFN-γ) overnight before harvest and storage at -80ºC. Pellets of ten million cells were processed per the manufacturer's instructions for the different lysis buffers: Thermo Scientific™ Mem-PER™ Plus Membrane Solubilization Buffer, Thermo Scientific™ Pierce™ IP Lysis Buffer, Thermo Scientific™ T-PER Tissue Protein Extraction Reagent, and Thermo Scientific™ Pierce™ GPCR Extraction and Solubilization Buffer.

Immunoprecipitation of MHC peptide complexes

W6/32 antibody was directly coupled to Pierce™ Protein A/G Magnetic Agarose Beads and Cytiva™ CNBr-activated Sepharose™ at room temperature for 1 hour. Lysates from 10 million cells were incubated with coupled supports for 2.5 hours at 4ºC. Beads were then collected, washed with PBS and water, and peptides were eluted at low pH (1% TFA) for 2 minutes at room temperature. Before clean-up, eluates were spiked with Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (PRTC) to evaluate peptide recovery. Clean-up was performed using a 10kD MWCO filter, C18 spin tips, or SDB spin tips. Finally, samples were dried down before resuspension in 0.1% formic acid in water before nanoLC-MS/MS analysis.

LC-MS/MS analysis

A Thermo Scientific™ Vanquish™ Neo UPLC system equipped with an IonOpticks™ Aurora™ Ultimate XT 25 x 75 cm Starter Pack (C18 column, heater, and temperature controller) coupled to a Thermo Scientific™ Orbitrap™ Exploris™ 480, Thermo Scientific™ Eclipse™ Tribrid™, or Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer was utilized for analysis of immunopeptidomics samples using a flow rate of 0.3 µl/min. and a gradient up to 120 minutes.

Data analysis

Data was analyzed using PEAKS® Studio 12.5 software with DeepNovo Peptidome workflow with peptide length 6-30, PSM-10lgP ≥ 20.0, PSM FDR 1.0%, and Peptide FDR 1.0%. Peptide recovery was calculated using quality control peptide standards and Skyline software.

Results

Workflow for isolation and analysis of MHC-I associated peptides (MAPs)

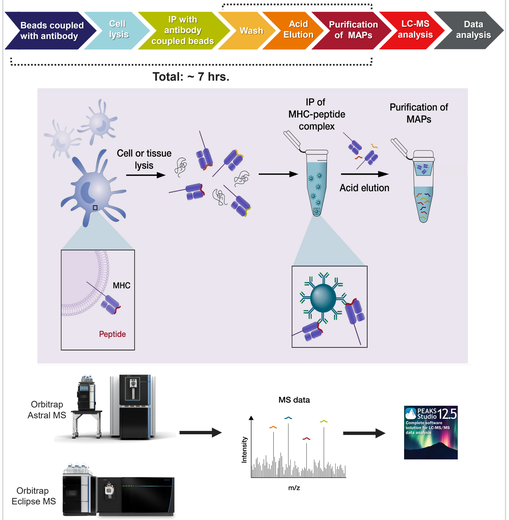

Figure 1. Immunopeptidomics workflow using magnetic agarose beads & MS analysis.

Table 1. Reagents, consumables, and software used in this study

	Reagents and Consumables
Cell Line	HCT116
Lysis buffer	Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit, Membrane Solubilization Buffer
IP Beads	Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose
IP Antibody	Invitrogen™ HLA-ABC Monoclonal Antibody (W6/32), Functional Grade
Clean-Up Tips	CDS Analytical™ Empore™ C18 Stage Tip
Internal Standard	Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture
LC-MS C18 Column	IonOpticks™ Aurora™ Ultimate XT (25cm x 75µm)
Data Analysis	PEAKS® Studio 12.5

MS evaluation of lysis buffers for immunopeptide enrichment

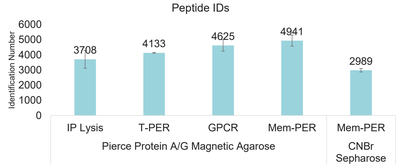

Figure 2. Four cell lysis buffers for IP from HCT116 cell lysates were compared using Pierce Protein A/G Magnetic Agarose beads and CNBr Sepharose. Resulting peptide identifications are shown with the Mem-PER solubilization buffer yielding the most peptides. All CVs were less than 10%, except IP Lysis Buffer (20%).

MS evaluation of IP support and cells with and without treatment

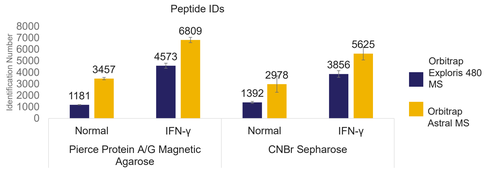

Figure 3. HCT116 cells +/- IFN-γ stimulation were subjected to IP using Pierce Protein A/G Magnetic Agarose and CNBr Sepharose.The elutions were run on Orbitrap Exploris 480 MS and the Orbitrap Astral MS. The resulting peptide IDs are shown with higher IDs in the IFN-γ samples with the magnetic beads and the Orbitrap Astral MS. Deeper coverage is achieved with Orbitrap Astral MS versus Orbitrap Exploris 480 MS due to higher instrument sensitivity for low-abundant peptides.

Evaluation of different clean-up formats

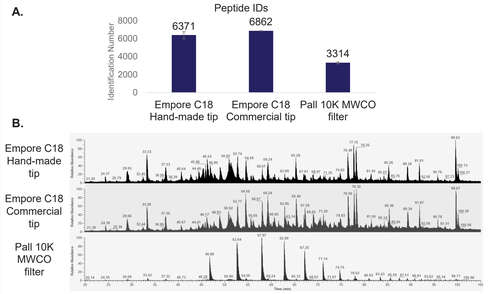

Figure 4. IPs were performed using IFN-γ-stimulated HCT116 cells and Pierce Protein A/G Magnetic Agarose, followed by cleaning the peptides using different methods. (A) C18 doubles peptide IDs compared to the 10kD molecular weight cut-off (MWCO) filter method. CVs were < 10%. (B) The resulting total ion chromatograms (TICs) are very similar for both of the C18 tips; however, PEG contamination is seen using the MWCO filter.

Evaluation of different clean-up methods in tip format

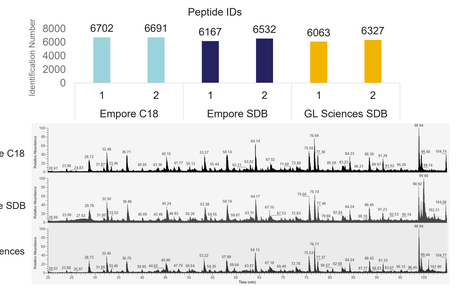

Figure 5. IPs were performed using IFN-γ-stimulated HCT116 cells and Pierce Protein A/G Magnetic Agarose, followed by cleaning up the peptides using different spin tips. (A) Empore C18 spin tips resulted in 5.2% and 7.5% higher peptide identifications on average than Empore SDB and GL Sciences SDB tips, respectively. (B) Very similar TIC profiles were obtained across 3 clean-up methods.

Assessment of peptide recovery using Pierce Peptide Retention Time Calibration Mixture (PRTC)

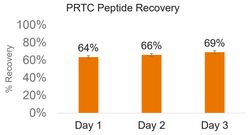

Figure 6. IP elution samples were spiked with PRTC before C18 spin tip clean-up, followed by LC-MS/MS analysis. Skyline software was used to calculate % recovery based on peak areas. Optimized C18 spin tip clean-up method resulted in > 60% recovery of 100fmol PRTC peptide standard.

Comparison of supports with optimized IP and clean-up method

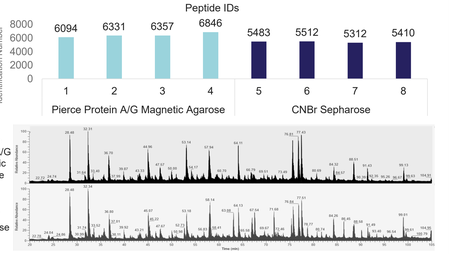

Figure 7. IPs were performed using IFN-γ stimulated HCT116 cells with Pierce Protein A/G Magnetic Agarose or CNBr Sepharose. (A) Protein A/G magnetic agarose IPs resulted in a 15% greater number of peptide IDs compared to the CNBr Sepharose IP method. (B) TIC showed very similar peptide elution profiles with both IP methods.

Reproducibility using Pierce protein A/G magnetic agarose for IP

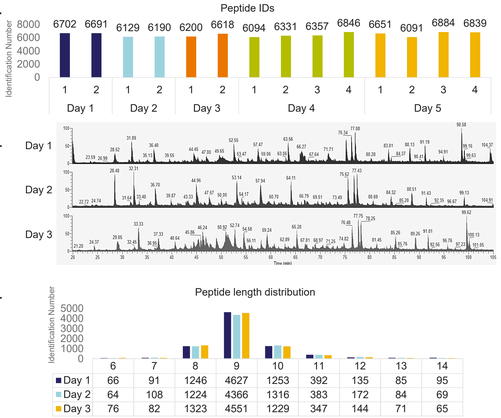

Figure 8. IPs were performed using IFN-γ stimulated HCT116 cells and Pierce Protein A/G Magnetic Agarose. (A) Peptide ID reproducibility between technical replicates over 5 days with 4.7% CVs. (B) Comparable chromatography is observed between days. (C) Peptide length distribution indicates 57% of peptide IDs for 9-mer immunopeptides.

Conclusions

- Pierce Protein A/G Magnetic Agarose enables reproducible enrichment of MHC-I complexes from HCT116 cells and are compatible with both manual and Kingfisher™ workflows. **(Poster WP543)**
- Quality control can be achieved using Pierce PRTC as an internal standard to determine clean-up efficiency and day-to-day reproducibility.
- Our optimized workflow enables high-quality, reproducible immunopeptidomics sample preparation within a standard workday using commercially available reagents.

Reference

1. Hesnard, L., Thériault, C., Cahuzac, M., Durette, C., Vincent, K., Hardy, M.-P., Lanoix, J., Lavallée, G. O., Humeau, J., Thibault, P., & Perreault, C. (2024). Immunogenicity of Non-Mutated Ovarian Cancer-Specific Antigens. Current Oncology, 31(6), 3099-3121. https://doi.org/10.3390/curroncol31060236

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