SAPrIm: a semi-automated protocol for immunopeptidomics of cells, tissues, and plasma using MagReSyn® Protein A

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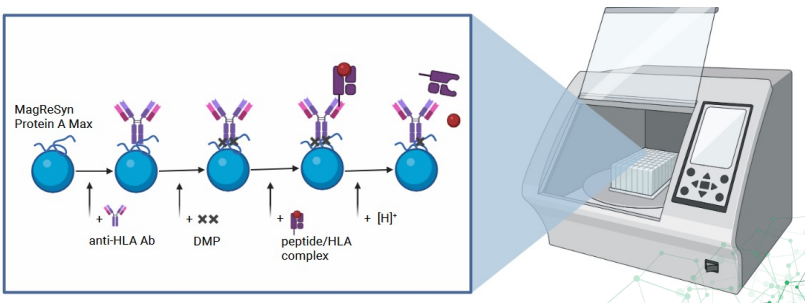
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Introduction

Immunopeptidome profiling refers to the global analysis of the Major Histocompatibility Complex class I (MHC-I) and class II (MHC-II) associated peptides. The MHC system known as the human leukocyte antigen (HLA) in humans is located on the short arm of chromosome 6. HLA bound peptides play a crucial role in the development of adaptive immune response in vertebrates. Comprehensive immunopeptidome profiling can enable novel solutions in personalized antitumor, antiviral, antibacterial and autoimmune disease immunotherapies. There are however several analytical challenges associated with immuno-peptidome profiling, as highlighted by the Human Immunopeptidome Project. These include peptide isolation uncertainty, the inability to analyze small amounts of biological material, low throughput, lack of sensitivity and reproducibility of mass spectrometry technologies, suboptimal identification rates, lack of experimental and computational standards, and the current lack of accessibility to large-scale community generated datasets.

We describe a semi-automated method for MHC molecule immunoprecipitation, covering all the steps from anti-MHC antibody cocktail load on high capacity MagReSyn® Protein A magnetic beads (crosslinking protein A and antibody for application to plasma), to the elution of purified MHC Class I and II complexes.

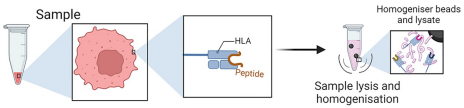
The method describes processing of up-to 12 samples in parallel but can be scaled to 96 samples without requirement for method re-optimization, and can be routinely automated on e.g. a KingFisher™ platform.



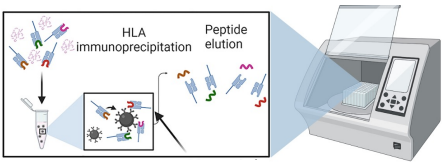
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Method: SAPrIm

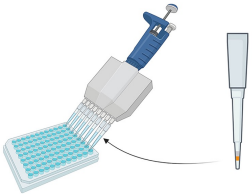
1. Sample lysis and homogenization



2. Peptide-HLA immunoprecipitation and acid elution using antibodies bound to MagReSyn® Protein A MAX.



3. Separation of HLA peptides from heavy chains and beta2-microglobulin using C18 or SDB-XC

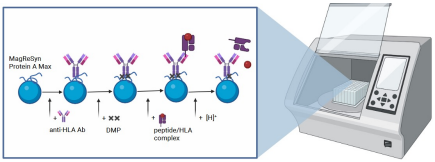


Method: SAPrIm 2.0

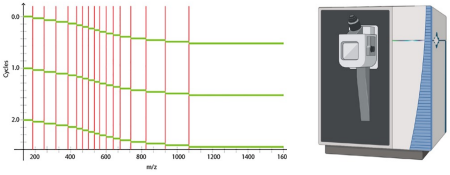
1. Plasma lysis and pre-clearance



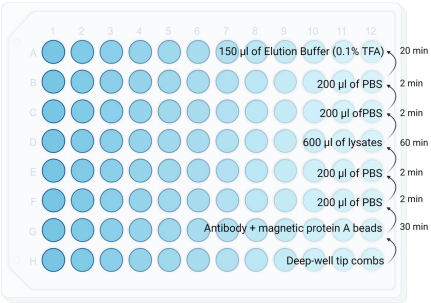
2. Peptide-HLA complex immunoprecipitation by using MagReSyn® Protein A MAX with crosslinked antibody



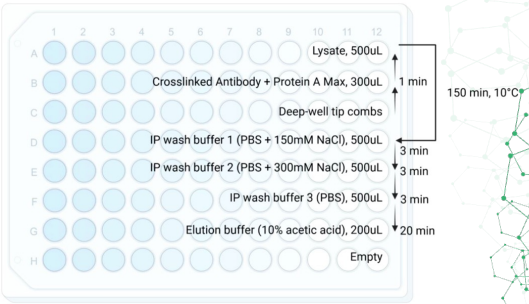
4. Mass spectrometry – DIA with variable m/z windows



KF Plate Layout: SAPrIm

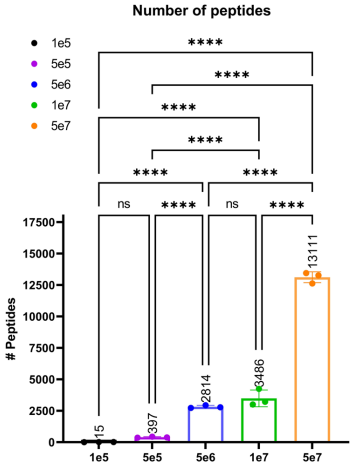


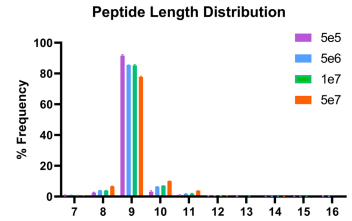
KF Plate Layout: SAPrIm 2.0

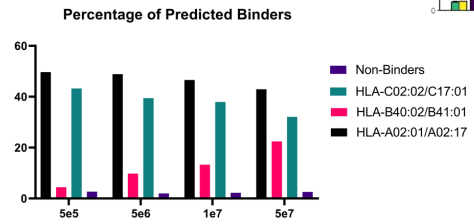


The original SAPrIm protocol by Lim *et al*. 2023, was readily adapted by Tanuwidjaya *et al*. 2025, for application to plasma by crosslinking the antibody to the MagReSyn® Protein A MAX magnetic beads. This prevents the antibody from being displaced when applying the beads to soluble HLA analysis in plasma. Steps 3 and 4 are common to both workflows. In addition, immunoglobulins present in plasma are simply pre-cleared via incubation with unconjugated MagReSyn® Protein A MAX beads.

Results: SAPrIm



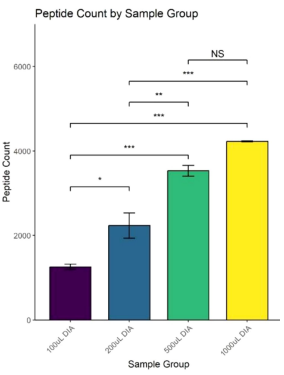


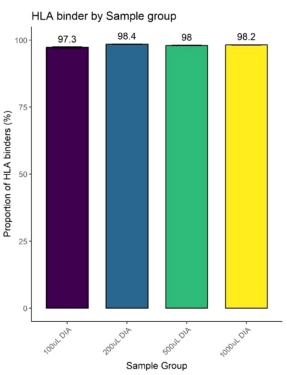


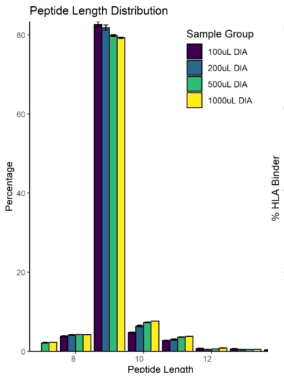
Immunopeptidomics analysis was performed on the MDA-MB-231 cell line for validation of the SAPrIm protocol. Cell input amounts tested varied from $1e^5$ – $5e^7$, with 13,312 unique HLA-I peptides being identified across all samples at 1% FDR. Immunopeptidome profiling of $1e^5$ cell pellets allowed for identification of only 15 peptides and was not used for further analysis.

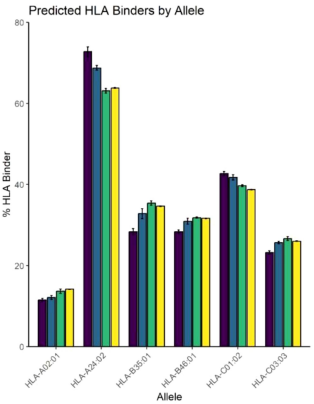
The SAPrIm 2.0 protocol was applied to 100 µl – 1 ml of plasma derived from the same sample pool.

Results: SAPrIm 2.0





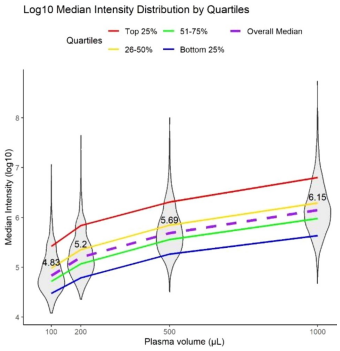


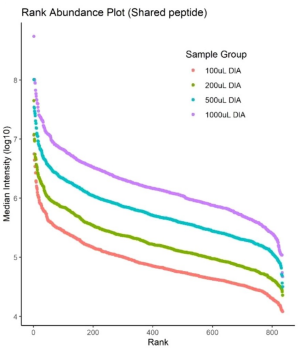


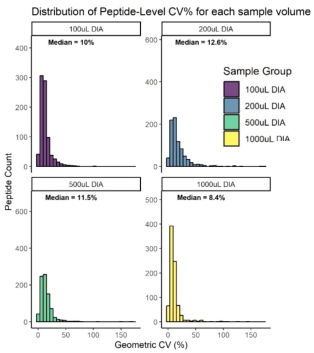
Using an experimentally-generated spectral library, 5,142 unique HLA-I peptides were identified at 1% FDR. While the 1 ml samples provide the highest peptide count, the increase between 500 µl and 1 ml is not statistically significant.

Both cell line and plasma datasets displayed a high proportion of 9mers across all input amounts as expected for HLA class I. Binding prediction analysis performed using NetMHCpan-4.1 on the identified HLA-I peptides, showed that over 95% were predicted to bind against the relevant HLA allotypes (those expressed on MDA-MB-231 cell line/plasma donor allotypes) for both datasets.

Results continued...







A panel of immunopeptides (peptides n = 835) were consistently quantified across all plasma samples, representing the core immunopeptidome that could be captured, and was used to evaluate the quantitative performance of SAPrIm 2.0. Peptides were ranked and stratified into quartiles based on their median intensities. High-abundance peptides (top 25% quartile) displayed a near-linear increase in intensity and the overall median showed a relatively consistent response with increasing volume of plasma. A high similarity in peptide rank distributions between plasma volume groups was observed and all input amounts displayed a peptide intensity Coefficient of Variation (CV) of below 13%.

MagReSyn® Protein A MAX: Cat# MR-PAM

Conclusions

SAPrIm reduces the complexity and preparation time for immunopeptidomics samples compared to current protocols. This semi-automated approach allows for the identification of ~400 – 13000 unique peptides from $5e^5$ – $5e^7$ cells. The SAPrIm 2.0 protocol, includes minor additions including antibody crosslinking to MagReSyn® Protein A MAX & plasma sample pre-clearance. Given the ease of accessing patient plasma compared to tissue, soluble HLA immunopeptidomics has recently become a topic of considerable interest. SAPrIm 2.0 enables comprehensive soluble immunopeptidome profiling using only 500µl of plasma, addressing previous challenges preventing widespread adoption of plasma immunopeptidomics.

References

1. Lim TCC, Goncalves G, Steele JR, Shameki T, Bramberger L, Jin D, Shabazy M, Purcell AW, Ramarathinam S, Stoychev S, Faridi P. SAPrIm, a semi-automated protocol for mid-throughput immunopeptidomics. Frontiers in Immunology. 14. 2023. doi.org/10.3389/fimmu.2023.1107576

2. Tanuwidjaya E, Lim TCC, Steele JR, Goncalves G, Woodhouse IB, Chang J, Ooi JD, Schittenhelm RB, Faridi P. SAPrIm 2.0: a semi-automated protocol for mid-throughput soluble HLA immunopeptidomics. Frontiers in Immunology. 16, 2025: doi.org/10.3389/fimmu.2025.1546629

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