

Mag-Net™ HP Kit for enrichment of extracellular vesicles from urine for automated HT proteomics



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Introduction

Urine is a source of diverse information related to a patient's health, and is gaining in popularity as a biofluid for clinical proteomics applications, especially since sampling is minimally invasive.

However, proteomics profiling of urine faces major hurdles due to the low protein concentration, interference from high salt and urea content, and the high physiological variability.

Extracellular vesicles (EVs) in urine are a potential source of valuable proteomics information as they are known to contain cell-specific proteins that can reflect the physiological or pathological state of a person.

Mag-Net™, when applied to enrichment of extracellular vesicles in urine, could potentially provide additional insights into the pathophysiology of various diseases. To this end we developed the Mag-Net™ HP Kit for urinary proteomics, suitable for enrichment from as little as 300 µl of urine.

The Mag-Net™ HP kit for urine proteomics is compared to the published urine-HILIC (uHLC) sample preparation method published by Govender *et al.* in 2023 using MagReSyn® HILIC.

To further assess the workflow for high-sensitivity applications, we use the 40 SPD Whisper Zoom method with an Aurora® Elite column.

Advantages of Mag-Net™ HP Technology for Urine Proteomics

Species agnostic

Low sample input

Automatable kit format

Increased proteome depth



Methods

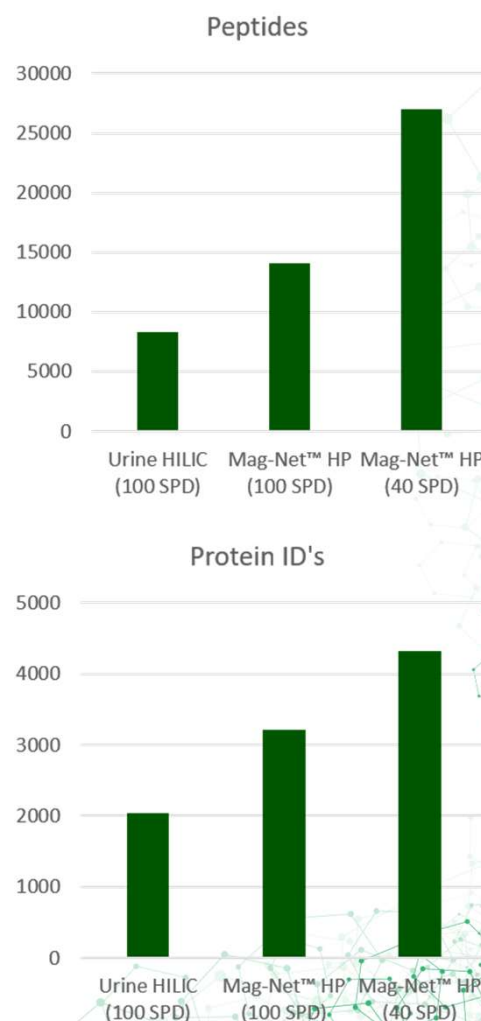
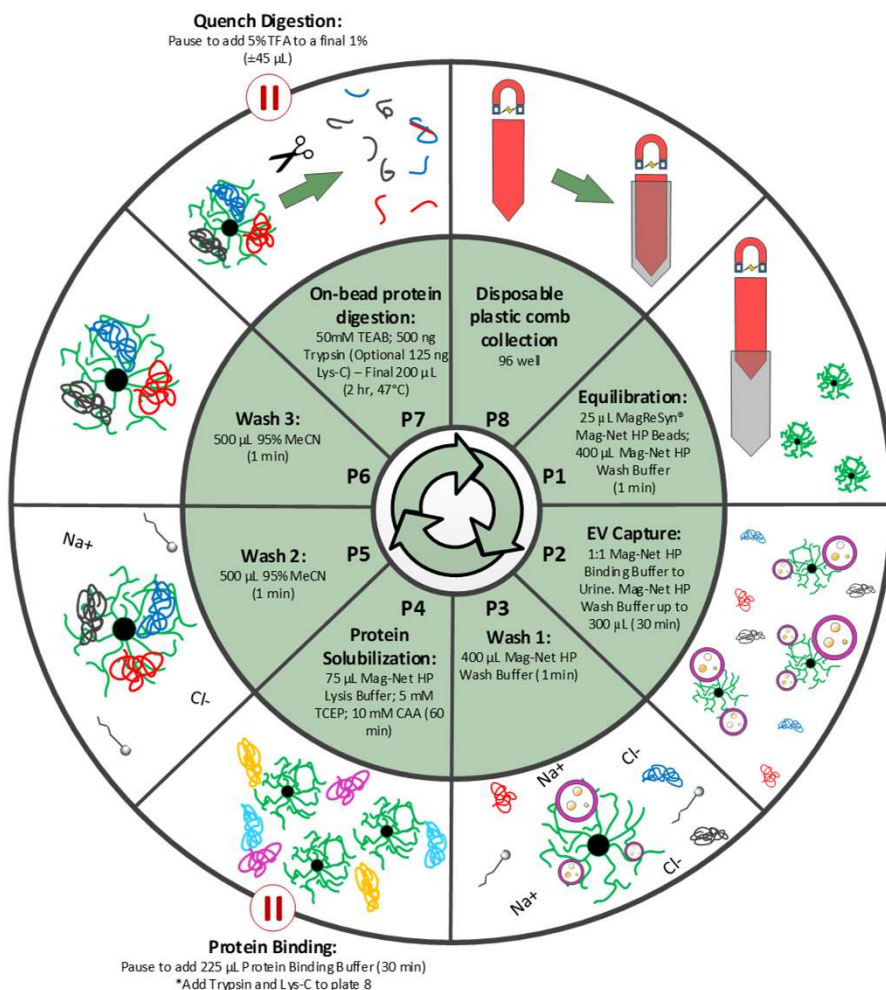
Urine for the Mag-Net™ and uHLC workflows were collected from a pool of healthy donors (4 people). First-morning, midstream, clean-catch urine was collected into sterile urine collection containers and transported immediately on ice to prevent degradation. Individual samples were centrifuged at 800× g for 10 min to remove debris, combined and then aliquoted and stored at –80 °C until required.

The Mag-Net™ HP workflow can be automated on a variety of magnetic bead, & liquid handling stations. In the current study the workflow was automated on a KingFisher™ Flex for high throughput sample preparation and analysis. KingFisher™ scripts for the Duo, Flex and Apex are available on request, please contact info@resynbio.com.

Peptides were vacuum dried and resuspended, of which 25% was loaded onto Evtips and immediately analysed on an Evosep One connected to a timsTOF HT (Bruker).

Results

The Mag-Net™ HP kit for urinary proteomics significantly increased the number of identified proteins and peptides compared to the published urine-HILIC workflow. The total protein identifications increased to over 3200 using Mag-Net™ HP from just over 2000 using the uHLC workflow at 100 SPD, and depth of coverage increased to over 4300 proteins when using a 40SPD Whisper Zoom method on the Evosep One fitted with the Aurora® Elite™ 15×75 CSI C18 UHPLC column.

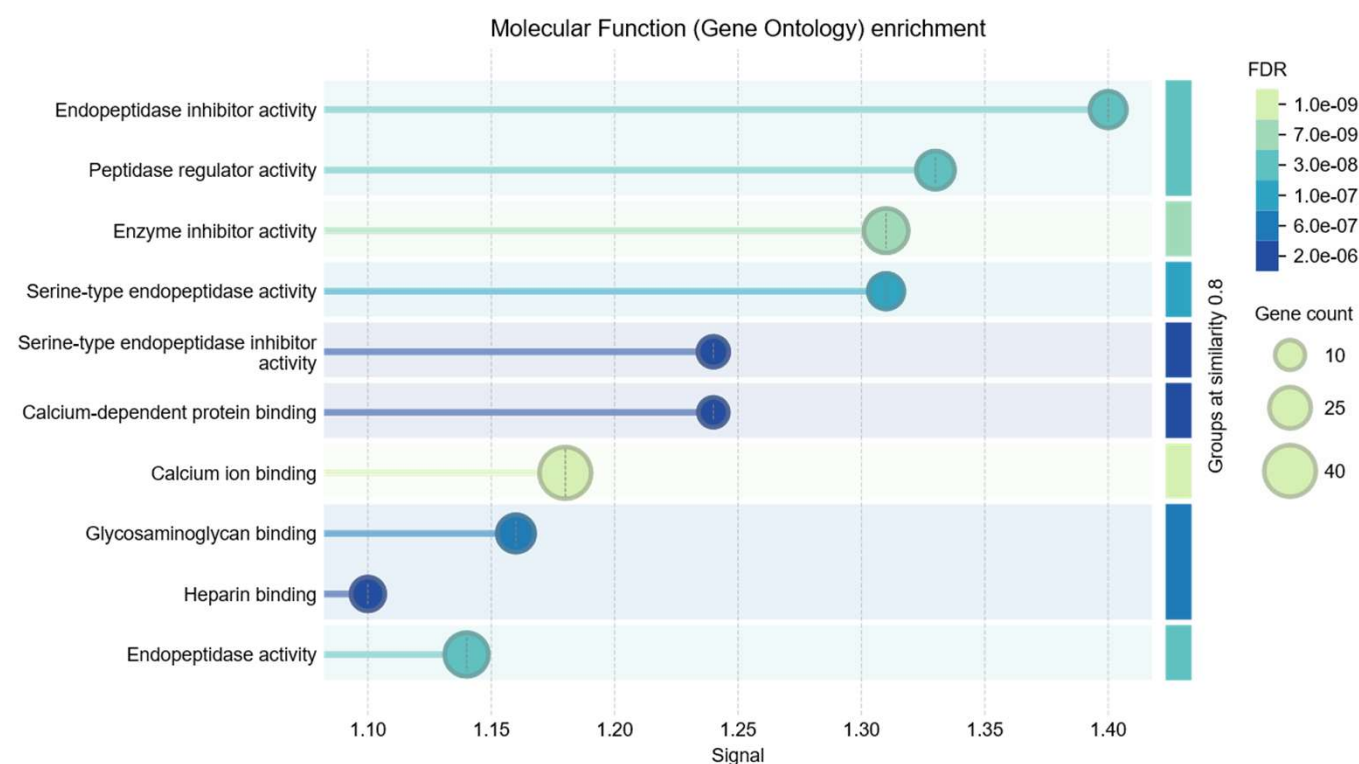
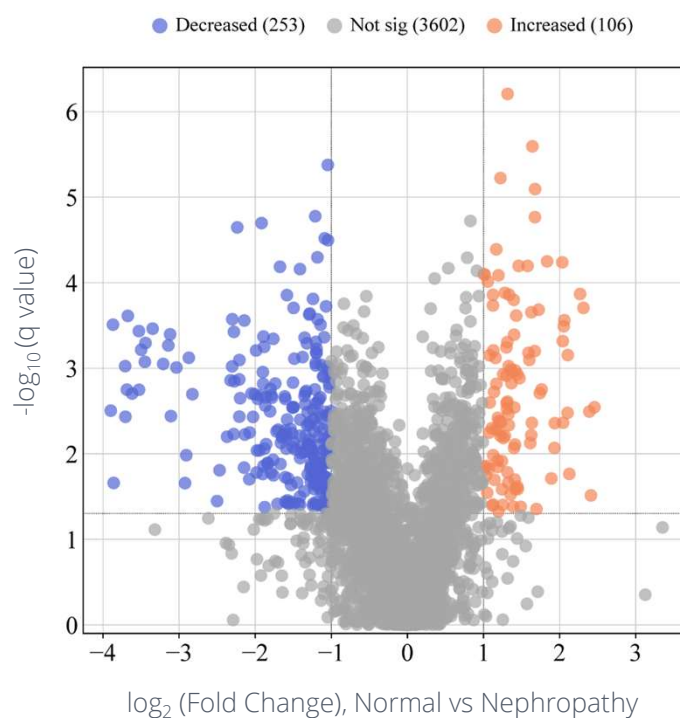


Results continued...

To demonstrate the application for biomarker discovery, the HP Kit was applied in a pilot study at the CSIR, for a cohort of HIV positive patients with and without kidney disease after ART treatment.

Across all pilot samples, the study identified 4,416 proteins and 30,800 peptides. In patients with nephrotoxicity, 359 proteins showed significant changes in abundance (FDR < 5%, fold-change > 2): 253 proteins were downregulated; 106 proteins were upregulated.

Novel nephropathy associated proteins were identified in the pilot and previously known pathways showed enrichment after the Mag-Net™ HP urine EV enrichment workflow.



Conclusions

The Mag-Net™ HP urine workflow allowed for deeper urinary proteome profiling than the current automatable published method urine HILIC, and significantly increased proteome coverage in a pooled sample from healthy participants.

Novel biologically relevant proteins were identified using the Mag-Net™ HP workflow in a pilot study on nephrotoxicity, showing utility of the workflow for discovery proteomics applications.

Acknowledgements



Product Information



Catalogue number: MR-MAGHP-96-URINE

The Mag-Net™ HP (High Performance) Kit for urine proteome profiling contains magnetic polymeric microparticle beads designed for the capture of membrane bound vesicles using the Mag-Net™ approach as described by the MacCoss Lab at University of Washington Department of Genome Sciences. The kit contains proprietary beads and buffers to achieve increased proteome coverage through the enrichment of membrane bound vesicles. The kit has been optimized for use with urine up to a volume of 300 µl per sample, which was found to be ideal based on in-house developments. Please contact us on info@resynbio.com if you require further information on this product.

References



1. Wu CC, Tsantilas KA, Park J, Plubell D, Sanders JA, Naicker P, Govender I, Buthelezi S, Stoychev S, Jordaan J, Merrihew G, Huang E, Parker ED, Riffle M, Hoofnagle AN, Noble WS, Poston KL, Montine TJ, MacCoss MJ. Enrichment of extracellular vesicles using Mag-Net for the analysis of the plasma proteome. *Nature Comms*. 16, 5447, 2025 Jul: doi: 10.1038/s41467-025-60595-7.



2. Govender IS, Mokoena R, Stoychev S, Naicker P. Urine-HILIC: Automated Sample Preparation for Bottom-Up Urinary Proteome Profiling in Clinical Proteomics. *Proteomes*. 2023 Sep; 28. doi: 10.3390/proteomes11040029

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