

Mag-Net™ HP and Evosep Eno enable deep plasma profiling suitable for high-throughput clinical proteomics



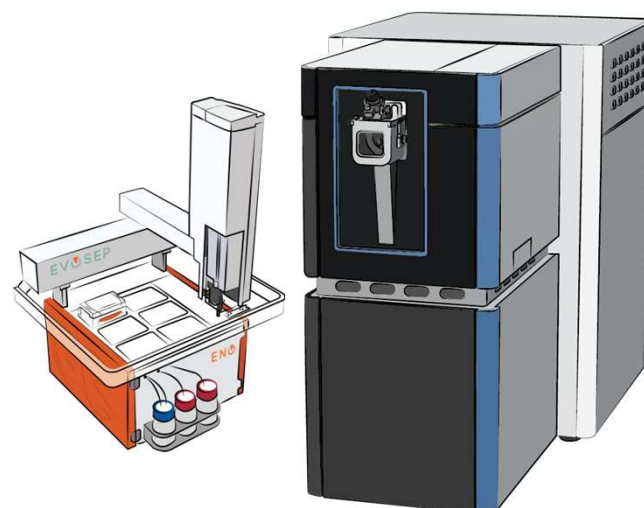
EVOSEP

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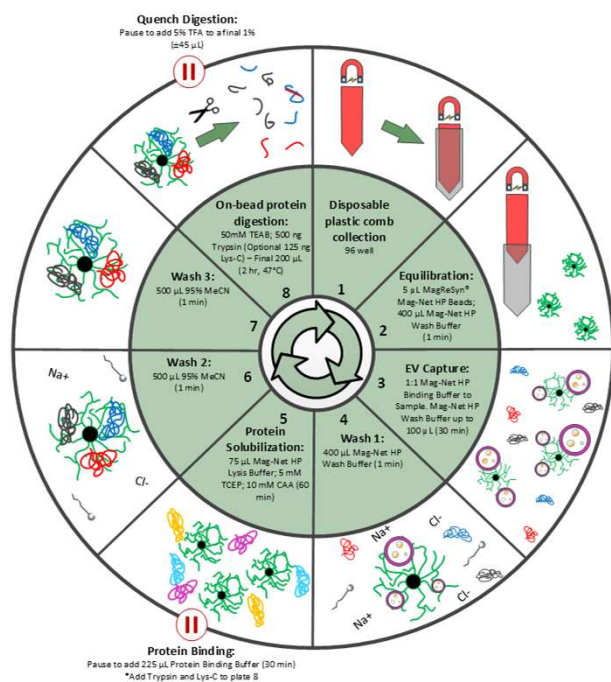
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Introduction

Large-scale clinical proteomics relies on the availability of standardized and robust sample preparation workflows. Plasma proteome profiling is challenging due to the large dynamic range of proteins present spanning 10-12 orders of magnitude. The Mag-Net™ workflow for deep plasma profiling as developed by Wu *et al.* 2023¹, relies on the enrichment of membrane bound vesicles, and concomitant reduction of abundant plasma proteins to reduce this dynamic range for deep plasma profiling. Our team at ReSyn Bio have developed an enhanced version of this workflow, termed Mag-Net™ HP (High Performance), that employs a multi-mode combination of beads, and streamlined workflow with reduced protocol steps. The simplified Mag-Net™ HP KIT can easily be automated on magnetic and liquid handling robotic systems. As an example, approximately 4000 protein groups (> 35000 precursors) were identified by automated Mag-Net™ HP on a Biomek i7, using the 200 SPD method on the new Evosep Eno LC coupled to an Orbitrap Astral MS, highlighting the utility of the workflow for large-scale clinical proteomics. We further present data using higher SPD on the new instrument by Evosep.



Methods



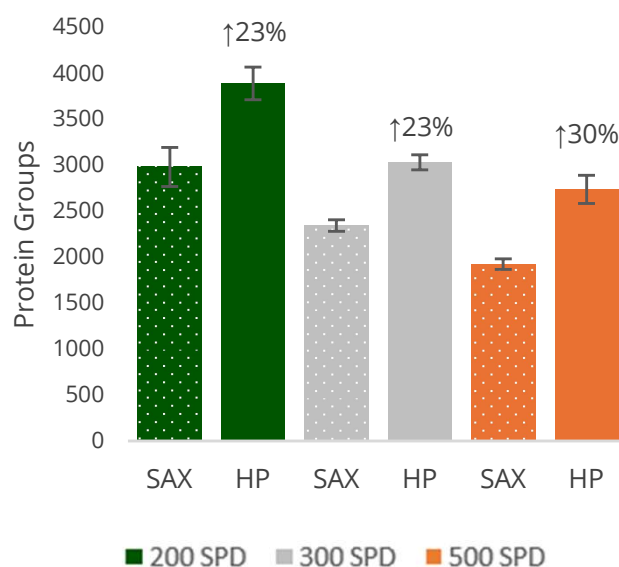
The Mag-Net™ HP workflow can be automated on a variety of magnetic bead, & liquid handling stations. In the current study the workflow was automated on a Biomek i7 from Beckman Coulter.

Plasma for the Mag-Net™ workflows presented here was collected from a pool of healthy donors, and processed based on the Early Detection Research Network (EDRN) standard operating procedure for collection of EDTA plasma². For comparison purposes, the Mag-Net™ SAX workflow was performed according to the optimized automated workflow as amended by Evosep using 4 µl of plasma and 1 µl of SAX beads, the Mag-Net™ HP workflow was performed according the KIT instructions as optimized by ReSyn Bio, with 5 µl of plasma and 5 µl of Mag-Net™ HP beads.

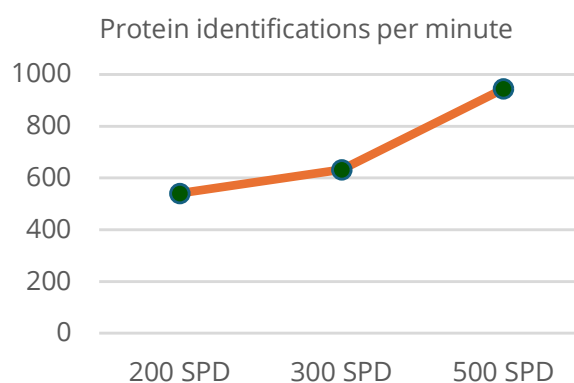
Resultant peptides were analyzed on an Evosep Eno coupled to an Orbitrap Astral mass spectrometer. The samples were analyzed with the 200, 300 or 500 SPD methods, and peptide LC on an EV-1182 column maintained at 40°C. DIA was performed using 3 Th precursor isolation windows with 7 millisecond accumulation time per window. Data was processed using DIA-NN v1.9.2 in library-free mode and analysis performed in R.

Results

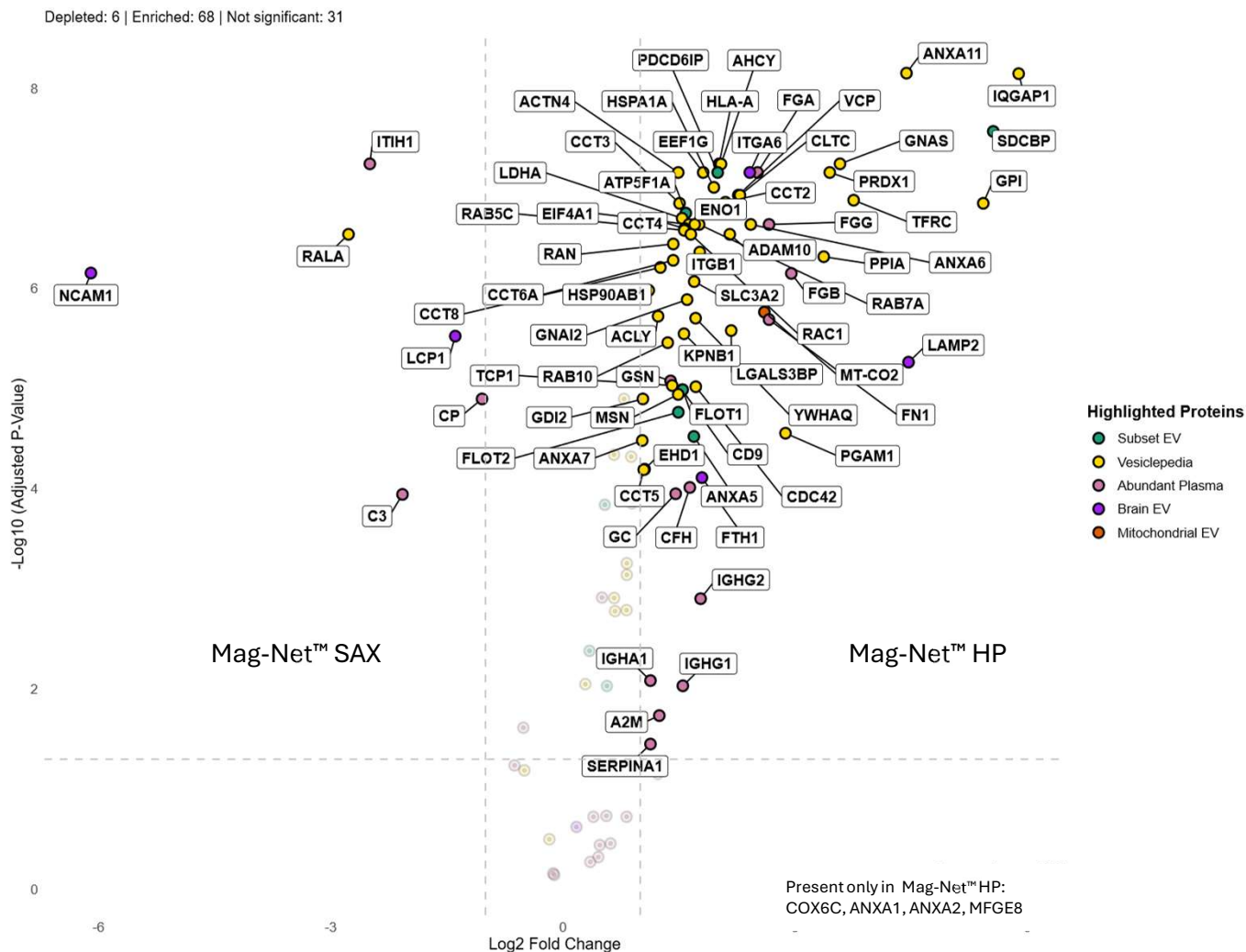
The new Mag-Net™ HP KIT increased the total proteome coverage in comparison to the optimized Mag-Net™ SAX workflow, with increased coverage of up to 30% in the 500 SPD method, identifying just under 3000 proteins, and 23% increase in total protein coverage for the 200 and 300 SPD methods, with ~4000 proteins identified using 200 SPD.



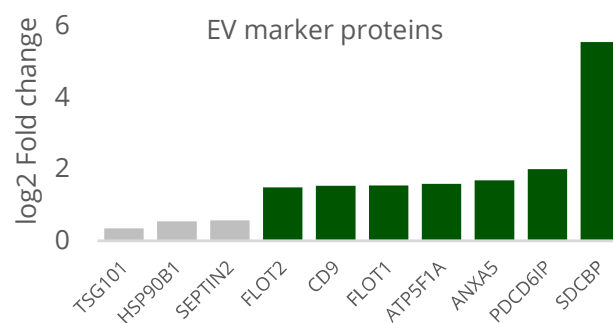
Normalized to runtime, the most significant gain in protein group identifications for Mag-Net™ HP was observed using the 500 SPD LC method, with protein identifications of almost 945 proteins per minute on the Evosep Eno, and over 500 proteins per minute on the 200 and 300 SPD methods.



Results continued...



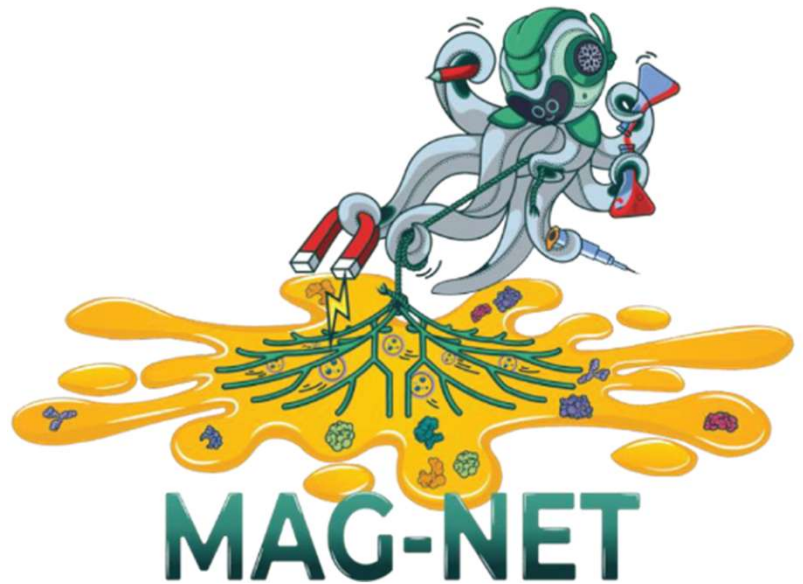
Quantitative differences in extracellular vesicle (EV) related marker proteins were compared using the data derived from the 200 SPD method for the Mag-Net™ SAX and HP workflows. Many of the top 100 EV-related marker proteins from Vesiclepedia are further enriched in Mag-Net™ HP when compared to SAX, albeit with some abundant plasma proteins showing the same trend. Specific exosomal markers (CD9, PDCD6IP, FLOT1, FLOT2 and SDCBP) showed a greater than 2-fold increase in abundance in Mag-Net™ HP samples. Interestingly, neuronal exosome marker NCAM1, while still detectable and enriched against plasma (data not shown), showed a greater than 64-fold decrease in Mag-Net™ HP processed samples. However, other brain-derived EV markers were enriched in Mag-Net™ HP when compared to Mag-Net™ SAX.



Conclusions

The Mag-Net™ HP KIT provides a simplified workflow for deep plasma proteome profiling that is easily automated and readily applicable for high-throughput clinical proteomics. Mag-Net™ HP shows an increase in EV derived marker proteins in comparison to the original Mag-Net™ SAX, attributing the mechanism of action to enhanced membrane vesicle isolation.

Acknowledgements



Product Information



Catalogue number: MR-MAGHP-96

The Mag-Net™ HP (High Performance) KIT 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 uses proprietary beads and buffers to achieve increased proteome coverage of plasma, and can provide over 50% increase in coverage over the traditional Mag-Net™ protocol. Using MagReSyn® SAX. The kit has been optimized for use with plasma, but can potentially be used for other biofluids (contact us for further information).

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. Mag-Net: Rapid enrichment of membrane-bound particles enables high coverage quantitative analysis of the plasma proteome. bioRxiv [Preprint]. 2024 Apr 2:2023.06.10.544439. doi: 10.1101/2023.06.10.544439.
2. Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, Rom W, Sanda M, Sorbara L, Stass S, Wang W, Brenner DE. Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group. J Proteome Res. 2009 Jan;8(1):113-7. doi: 10.1021/pr800545q.

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