Nanomics

Application Note

Establishing a Robust and Scalable Workflow for Plasma EV Protein Profiling with Proteonano™

Nanomics Biotechnology BD@nanomics.bio

Keywords:
Plasma, EVs, Proteonano™ EV Proteome Kit, Proteonano™ Lite Suite, Proteomics, Mass Spectrometry

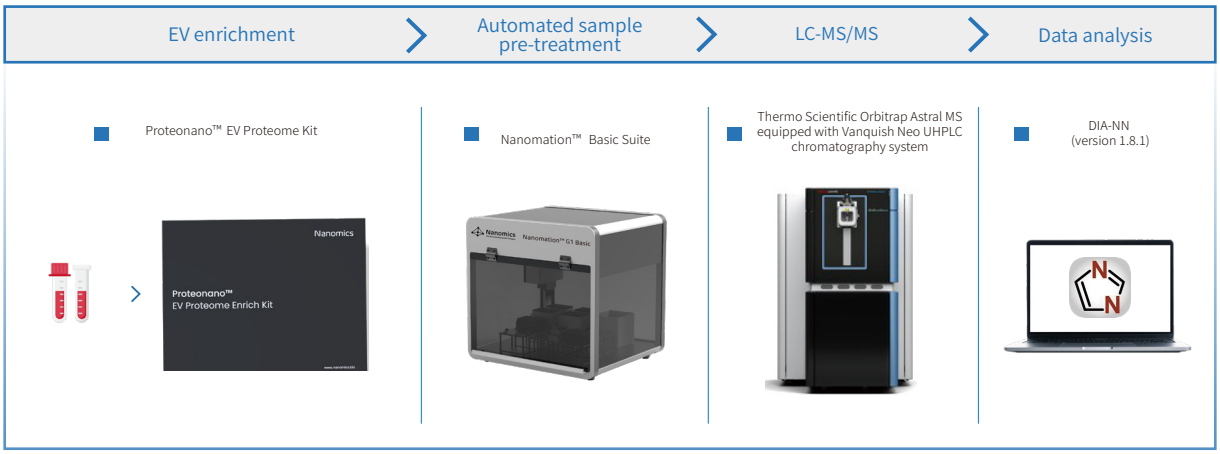
Objective:
A standardized workflow for EV protein detection and analysis in plasma has been developed using the Proteonano™ ultraplex proteomics platform in combination with mass spectrometry technology

Introduction

EVs are bioactive vesicular substances that carry nucleic acids, proteins, lipids, and metabolites. As key mediators of intercellular communication, they influence various aspects of cell biology. However, the heterogeneity in the origin and molecular composition of vesicles secreted by cells poses challenges for the study and application of EVs.

EVs have significant research and application potential in disease mechanisms, clinical diagnostics, targeted therapy, and drug delivery. In disease research, they help explore the cancer and cardiovascular diseases. As nanoscale biomarkers in liquid biopsies, EVs show great promise in clinical diagnostics. Their heterogeneity and role in specific signaling pathways make them key in cancer targeted therapies. Additionally, EVs serve as effective autologous carriers for precise drug delivery [1,2].

EV isolation and purification are crucial for studying their biological functions and proteomics. Common methods include ultracentrifugation, kit-based extraction, PEG precipitation, immunoaffinity, and TiO2 enrichment. Ultracentrifugation is considered the "gold standard" [3] due to its simplicity and lack of complex sample preparation.



Figuer 1: MS Combined with Proteonano™ Ultraplex Proteomics Platform for Mouse Blood Sample Proteomics Analysismics Analysis

However, it requires large sample volumes, making it unsuitable for small sample studies. The process is also time-consuming and labor-intensive, raising research costs.

To address these challenges, magnetic nanoparticle-based EV extraction has gained attention. This method significantly reduces sample volume requirements, needing only 50 µL of serum or plasma, and 1 mL of urine, cerebrospinal fluid, or bronchoalveolar lavage. The extracted EV proteins can be used for high-throughput proteomics. However, current magnetic bead technologies have limitations, including incomplete EV protein enrichment, data gaps, inconsistent preprocessing, and lack of standardization. These issues result in low protein data coverage and poor reproducibility, affecting data accuracy and reliability.

LC-MS/MS	Kit	Sample	Sample volume (µL)	Sample loading amount (ng)	Gradient (min)
Orbitrap Astral	Proteonano™ EV Proteome Kit	Plasma	40	200	8

Table: Experimental parameters for EV proteome

Results and Discussion

Proteonano™ platform performance for EV proteins in plasma samples

Plasma EV Protein Enrichment Efficiency

Using the Proteonano™ EV Proteome Kit to enrich EV proteins from healthy human plasma, electron microscopy (TEM) imaging shows significant enrichment effectiveness. The magnetic beads in the EV Proteome Kit are coated with exosome particles that are uniform in size and intact in shape (approximately 30-150 nm in diameter). The background contains minimal impurities, indicating that the EV Proteome Kit effectively reduces interference from non-target proteins and minimizes non-specific binding during the isolation process (see Figure 2).

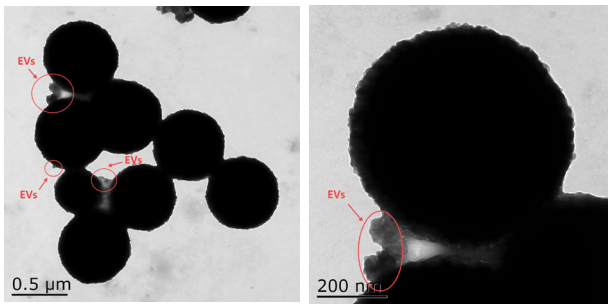


Figure 2: TEM image of plasma EV proteins enriched using the Proteonano™ EV Proteome Kit.

Protein Identification and Dynamic Range

The Proteonano™ EV Proteome Kit and Lite Suite high-throughput proteomics system enable deep protein coverage with ng-level protein samples. Mass spectrometry data is collected using the next-generation Orbitrap Astral high-resolution spectrometer coupled with the Vanquish NEO liquid chromatography system. Peptides are dissolved in buffer and injected via the auto-injector, with 200 ng of peptides loaded for separation. An 8-minute gradient is used, and data is acquired in DIA mode (see Figure 1, Table 1).

The results show that the Proteonano™ Ultraplex Proteomics Platform identified 5092 proteins in plasma , including 2,046 extracellular vesicle EV proteins. This demonstrates a significant improvement in protein identification with the Proteonano™ platfotm for plasma EVs (see Figure 3).

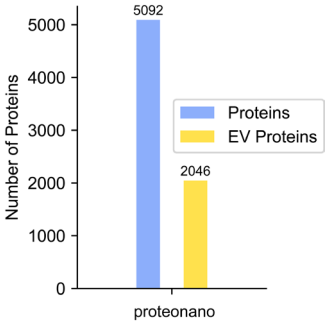


Figure 3: Number of EV proteins identified using the Proteonano™ EV Proteome Kit.

Coverage of EV Proteins in the ExoCarta Database

The EV protein data from the Proteonano™ EV Proteome Kit and commercial kits were compared with the 5,191 proteins listed in the ExoCarta database. The Proteonano™ EV Proteome Kit covered up to 39.4% of the database proteins, while commercial kits covered around 23%. This highlights the strong specificity of the Proteonano™ EV Proteome Kit for enriching plasma EV proteins (see Figure 4).

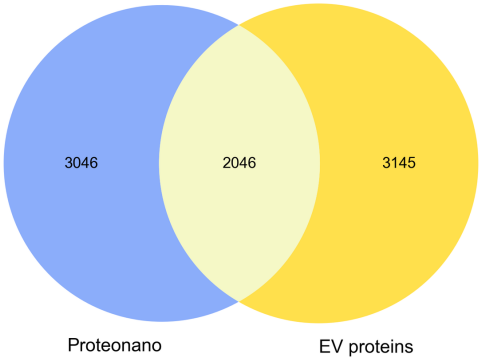


Figure 4: Overlap of Enriched Plasma EV Proteins with ExoCarta Database Proteins

Coverage of Top 100 EV Proteins in ExoCarta Database

A comparison of EV proteins enriched using the Proteonano™ EV Proteome Kit with the Top 100 EV proteins in the ExoCarta database showed that Proteonano™ detected more Top 100 proteins than commercial kits. It effectively captured exosome-specific proteins often missed by traditional methods, demonstrating higher sensitivity (see Figure 5).(see Figure 4).

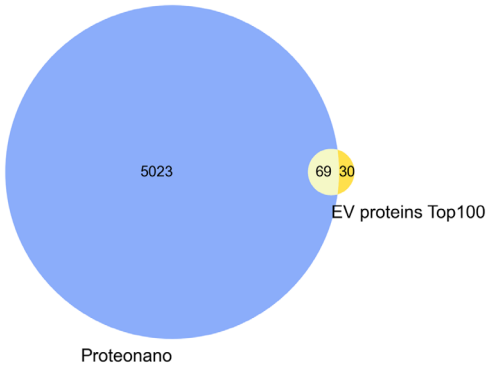


Figure 5: Overlap of Enriched Plasma EV Proteins with

Coverage of Surface550 EV Proteins

Comparison of Proteonano™ EV Proteome Kit with Vesicode@Surface550 EV protein data (Secretech Inc) shows that Proteonano™ detects approximately 40 more Surface550 proteins than commercial kits. This makes it a superior choice for plasma EV protein analysis, offering significant potential for EV proteomics research, disease diagnostics, molecular mechanism studies, and therapeutic target discovery (see Figure 6).

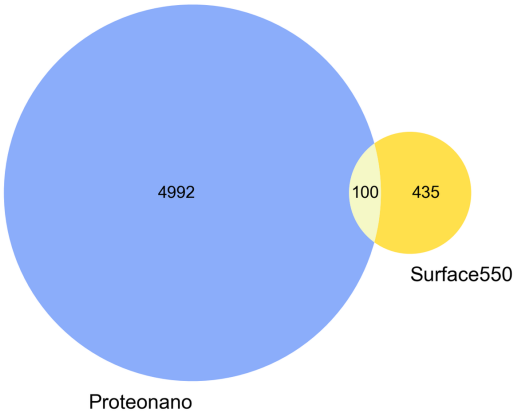


Figure 6: Overlap with Vesicode@Surface550 EV Proteins

Enrichment analysis

Enrichment analysis indicated that the identified plasma proteins were predominantly associated with B cells, platelets, and intestinal tissue, highlighting the potential of plasma proteomics to serve as a surrogate for detecting tissue-specific damage or pathological conditions

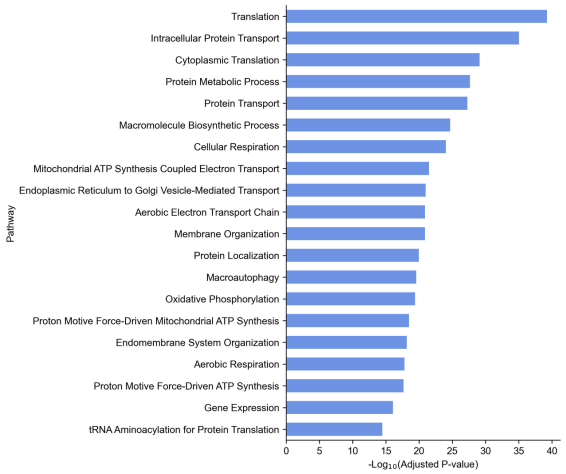


Figure 7: Proteins measured byProteonano™ Platform mapped by tissue distribution

Pathway enrichment analysis revealed significant involvement of protein metabolism and transport processes, underscoring the potential of plasma proteomics to capture dysregulated pathways associated with disease pathogenesis

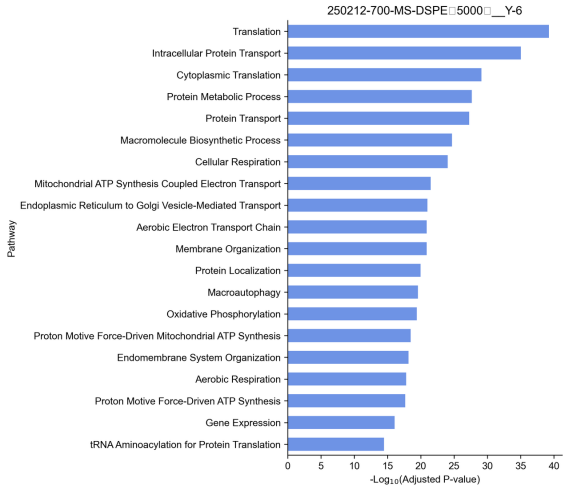


Figure 8: Proteins measured byProteonano™ Platform mapped by signaling pathways

Among the enriched cellular components, the numbers of associated proteins were 1164 for the cytoplasm, 1046 for extracellular vesicles, 265 for the cell membrane, and 229 for mitochondrion. (Figure 9)

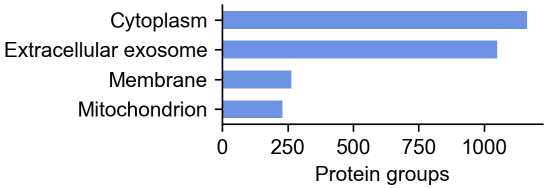


Figure 9: Proteins measured byProteonano™ Platform mapped by subcellular localization

Conclusion

This study evaluates the performance of the Proteonano™ Ultraplex Proteomics Platform, developed by Nanomics Biotech, for plasma EV proteomics.

(1) Superior Analytical Performance: The Proteonano™ platform outperforms commercial kits in plasma EV proteomics, detecting more EV proteins with higher sensitivity, as demonstrated by comparisons with ExoCarta Top 100 and Surface550 datasets.

(2) Efficient Target Protein Capture: Its optimized exosome isolation technology efficiently extracts extracellular vesicles (EVs) from plasma while minimizing loss. By reducing non-target protein interference, the platform significantly increases EV protein yield, making it a highly specific and reliable tool for exosome isolation.

(3) High Reproducibility and Standardization: The platform integrates automation and rigorous data analysis workflows, ensuring consistent and reproducible results.

In summary, the Proteonano™ platform provides a powerful and reliable solution for plasma Ev proteomics. Its robust performance and high specificity make it a strong alternative for proteomic analysis of biological samples, contributing to advancements in proteomics research.

Reference

[1] Wu Y, Deng W, Klinke II D J. EVs: improved methods to characterize their morphology, RNA content, and surface protein biomarkers[J]. Analyst, 2015, 140(19): 6631-6642.
[2] Shao H, Im H, Castro C M, et al. New technologies for analysis of extracellular vesicles[J]. Chemical reviews, 2018, 118 (4): 1917-1950.
[3] Matsumoto Y, Kano M, Akutsu Y, et al. Quantification of plasma EV is a potential prognostic marker for esophageal squamous cell carcinoma[J]. Oncology reports, 2016, 36(5): 2535-2543.
[4] Zhao B, Gao X, Ouyang X, et al. Proteonano: a novel deep proteomics platform with 1000-plex profiling capacity and picogram sensitivity and its application in diabetic kidney disease[J]. bioRxiv, 2023: 2023.09. 12.556305.

Nanomics Biotechnology
Email：haowu@nanomics.bio
Website：www.nanomics.bio