



# Proteonano™ and Evosep Integration for Automated High-Throughput Proteomics Workflow

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## Background

The Proteonano™ Ultraplex Proteomics Platform, developed by Nanomics Biotech, is a highly automated and standardized system for proteomic sample preparation. This Platform comprises the Proteonano™ Kit reagents and the Nanomation™ G1 automation equipment, specifically designed to address the challenges encountered in mass spectrometry analysis of low-abundance proteins in plasma samples. The Proteonano™ Kit series utilizes multi-affinity superparamagnetic nanoprobe developed by Nanomics Biotech to enrich low-abundance proteins within biofluidic sample.

The Evosep One is a high-throughput, highly stable, and ultra-low contamination liquid chromatography system designed for mass spectrometry-based proteomics research. Paired with the Evotip, which enables online desalting, the Evosep One enhances system stability. The system features 15 pre-configured methods for analyzing a variety of biological samples, including plasma, tissue, and single-cell samples. It is well-suited for clinical or large-scale proteomics studies.

In large-scale proteomics experiments, issues such as throughput, sample processing consistency, and batch effects are common challenges. Existing experimental protocols and automation platforms often struggle to address all these technical issues simultaneously.

Nanomics Biotech Proteonano™ platform enables high-throughput plasma proteomics sample preparation,

automating steps up to enzymatic digestion or desalting prior to mass spectrometry. Both Proteonano and Evosep are designed for large-scale proteomics studies, offering standard, high-throughput, and automated processes. Combining these platforms allows seamless transition of peptides into the Evotip for desalting, followed by automatic injection into the Evosep system for mass spectrometry.

The integration of the Proteonano and Evosep platforms into a unified automated workflow provides exceptional throughput and stability for large-scale proteomics studies, supporting mass spectrometry-based proteomics for cohort sizes of up to thousands.

In this report, we use the Proteonano™ Ultraplex Proteomics Platform developed by Nanomics Biotech ([www.nanomics.bio](http://www.nanomics.bio)) combined with advanced mass spectrometry technology to apply SP3 pre-treatment to biological samples for proteomics analysis, develop SP3 proteomics application scenarios for biological samples and establish standard analysis processes.

## Key Instrument and Software

### Protein Enrichment Reagents and Automated Pre-treatment System

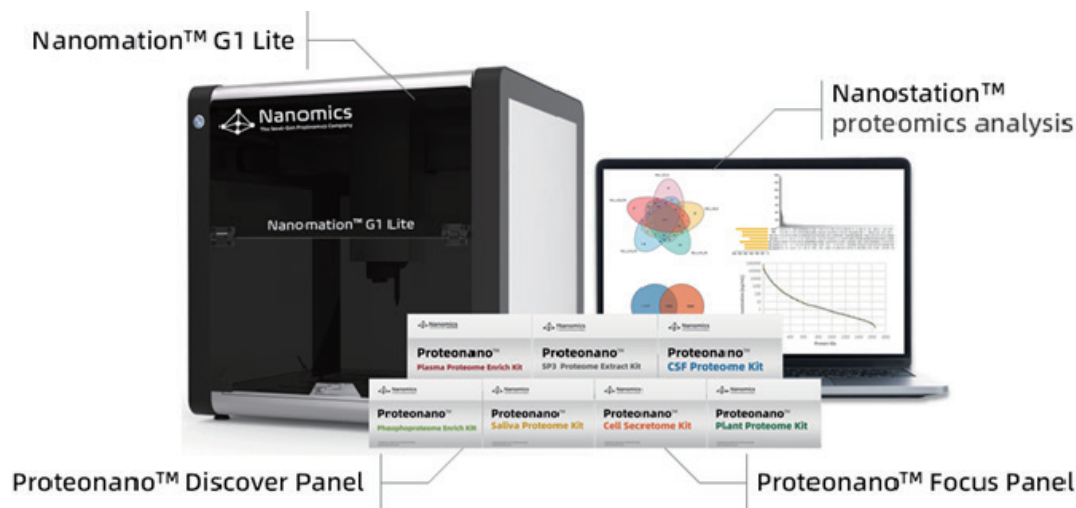
- Proteonano™ Plasma Proteome Enrich Kit (Nanomics Biotech, Hangzhou, China)
- Proteonano™ Ultraplex Proteomics Platform (Nanomics Biotech, Hangzhou, China)
- Trypsin (V5111 Promega Corporation, Madison, WI, USA)

## Mass Spectrometer & Liquid Chromatography

- Evosep One (EVOSEP, Odense, Denmark)
- timsTOF Pro 2 (Bruker Corporation, Billerica, MA, USA)

## Data Analysis Software

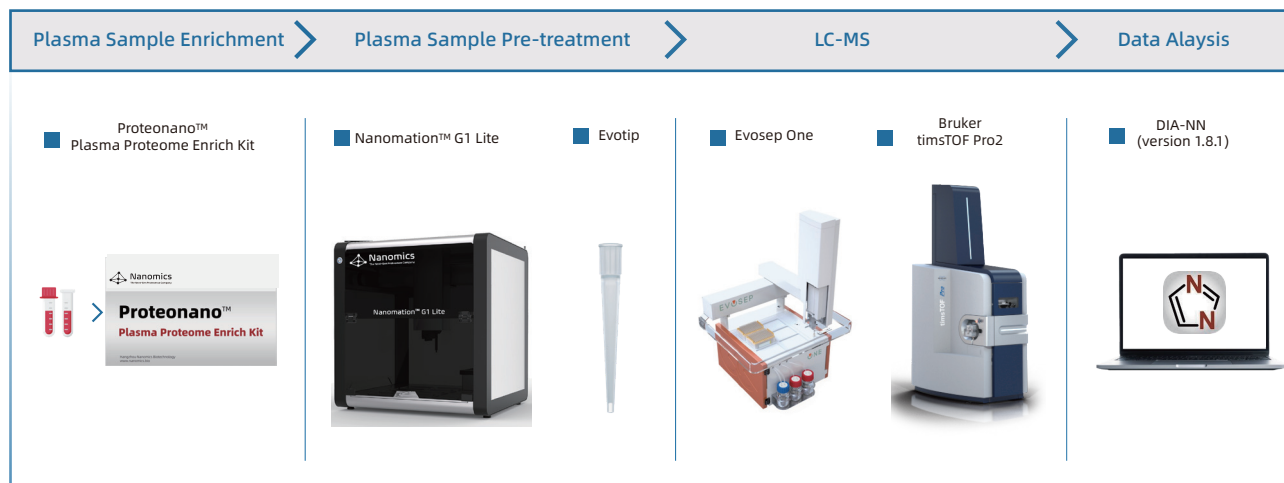
- DIA-NN (version 1.8.1)
- Uniprot Reviewed (Swiss-Prot) (20,422 Entries)



**Figure1:** Proteonano™ Lite suit High-Throughput Proteomics Analysis System

## Experiment Procedures

Using the Proteonano™ Ultraplex Proteomics Platform for automated enrichment of low-abundance proteins in plasma samples, specifically from healthy pool plasma. Subsequently, the Evosep One system, coupled with the timsTOF Pro2, performs standard gradient mass spectrometry analysis. The overall process is outlined in the schematic diagram below:



**Figure2:** Experimental Workflow of Proteonano™ Platform Lite suit Combined with Evosep one Platform

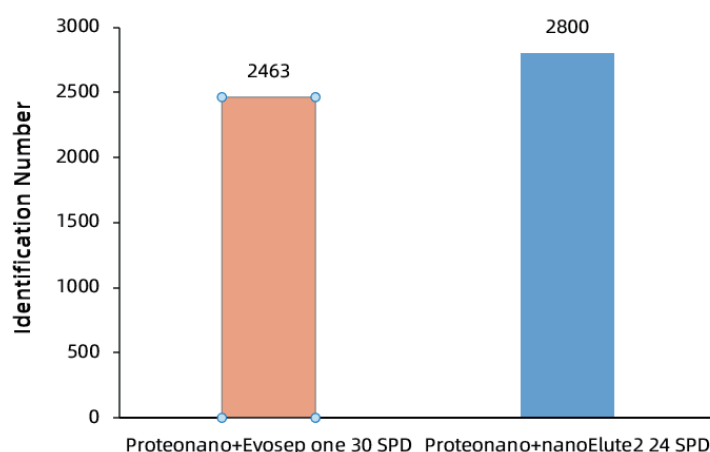
Kit	Pre-treatment	LC	MS	Sample	Injection volume (ng)	Gradient (SPD)
Proteonano™ Plasma Kit	Nanomics Lite	Evosep One	TimsTOF Pro 2	Human mixed plasma	300	30
		nanoElute® 2				24

**Table 1:** Reagent Kits and Sample Information Used in the Experiment

## Performance of Plasma Proteome Identification Using the Combined Proteonano and Evosep Platforms

### 1. Identification Number

Using the Proteonano™ platform Lite Suit, low-abundance protein enrichment was performed on 40 µL of healthy pooled plasma samples. Subsequent mass spectrometry analysis was conducted with the Evosep One system coupled with the timsTOF Pro 2. The results demonstrated the detection of 2,463 proteins with a 300 ng sample amount under a 30 SPD gradient condition. This is comparable to the identification of approximately 2,800 proteins using the same samples on the timsTOF Pro 2 with a 24 SPD (60 min) gradient [1].



**Figure3:** Number of plasma protein groups identified on the Proteonano and Evosep combined platform

### 2. Coverage of HPPP (Human Plasma Proteome Database) ratio

The Proteonano™ Plasma Proteome Enrich Kit is designed to enrich low abundance proteins in human plasma samples, allowing for the identification of a large number of proteins and the discovery of new proteins when combined with mass spectrometry. When proteins identified using the Proteonano™ platform Lite Suit in conjunction with the Evosep platform were compared to the Human Plasma Proteome Project (HPPP) database, it was found that the timsTOF Pro 2 detected proteins covering 1,733 proteins from the HPPP database, with 50% of these proteins having concentrations below 10 ng/mL. Additionally, 766 proteins were identified beyond the HPPP database.



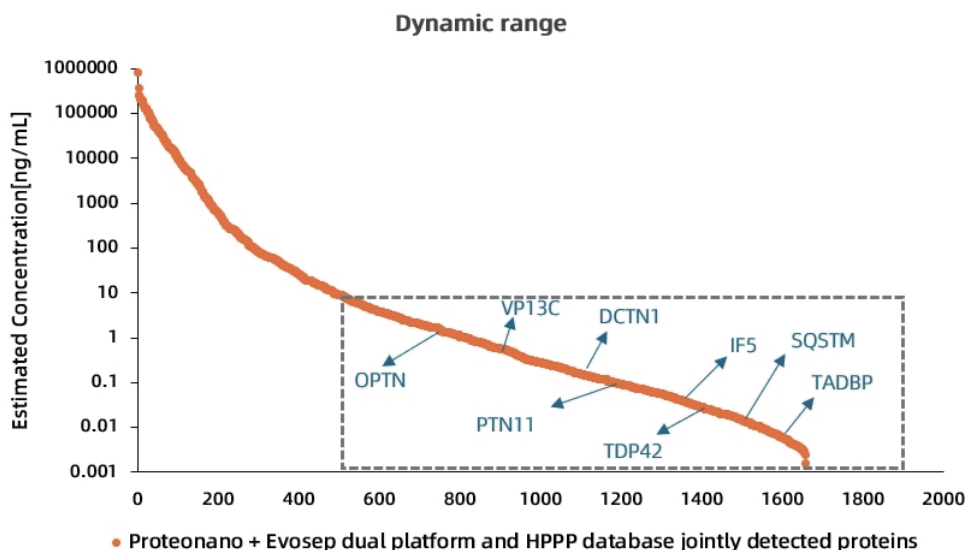
**Figure4:** Proteonano and Evosep combined platform to detect the number of protein groups coincident with HPPP

### 3. Dynamic range

Additionally, the proteome dynamic range detected in this experiment spanned over 9 orders of magnitude, representing



nearly a 10,000-fold increase in identification depth compared to traditional methods. As shown in Figure 5, several proteins associated with aging and neurodegenerative diseases such as Alzheimer's disease were found to have concentrations below 10 ng/mL.



**Figure5:** Depth of identification of Proteonano and Evosep dual-platform proteins

## Conclusion

This study integrates the Proteonano™ platform Lite Suit and the Evosep one platform for high-throughput proteomics, achieving automated high-throughput analysis of plasma samples and evaluating the performance of the combined platforms. The conclusions are as follows:

- (1) Outstanding Performance: The combination of the Proteonano™ platform Lite Suit and the Evosep one platform demonstrates excellent performance. Plasma samples enriched with the Proteonano™ Plasma Proteome Enrich Kit and processed using both platforms can identify over 2,400 proteins, showcasing superior protein identification capabilities.
- (2) Increased Depth: The Proteonano™ platform Lite Suit and Evosep one platform combination offers enhanced coverage, with a dynamic range exceeding 9 orders of magnitude.
- (3) High Standardization: The combined use of the Proteonano™ platform Lite Suit and Evosep one platforms ensures high standardization. Automated control of the entire experimental workflow, including sample preparation, desalting, and mass spectrometry analysis, guarantees the stability of results in large-scale experiments.

In summary, the integration of the Proteonano and Evosep platforms for plasma sample processing provides exceptional throughput and stability for proteomics studies, supporting mass spectrometry-based proteomics for large-scale cohorts of up to thousands of samples.

## References

- [1]. Sibowang, xiehua ouyang, haowu. Performance of the Proteonano™ Platform on Different Mass Spectrometers.[AN]

