Nanomics

Application Note

Proteomic Analysis of Cerebrospinal Fluid Using the Proteonano™ Ultraplex Proteomics Platform

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Keywords:
Cerebrospinal Fluid Proteomics, Proteonano™ CSF Proteome Kit, Proteonano™ Ultraplex Proteomics Platform, Orbitrap Astral

Objective:
We've developed a standardized cerebrospinal fluid protein analysis workflow using the Proteonano™ Ultraplex Proteomics Platform combined with Orbitrap Astral.

Introduction

Cerebrospinal fluid (CSF) is a clear, colorless fluid found in the brain' s ventricles and the subarachnoid space, serving essential functions such as protecting, supporting, and nourishing the brain and spinal cord[1]. The composition of CSF is relatively stable, including proteins, glucose, chloride, and enzymes, which help maintain its physiological roles. Key proteins in CSF include total protein (TP) and trace albumin (mALB). TP is commonly used to aid in diagnosing conditions like central nervous system inflammation, radiculopathy, and spinal canal obstructive disorders, as well as distinguishing between bacterial and non-bacterial infections[2]. mALB, primarily derived from the blood-brain barrier, is seen as an important marker for evaluating the barrier's integrity[3]. Therefore, accurate protein detection in CSF is crucial for clinical diagnostics.

Proteomics is a scientific approach that studies the whole proteins within a sample, examining aspects such as protein expression, translation, and interactions at the cellular, tissue, and organ levels[4]. This field includes both quantitative and non-quantitative techniques, with quantitative proteomics being the more widely used. Quantitative proteomics can be

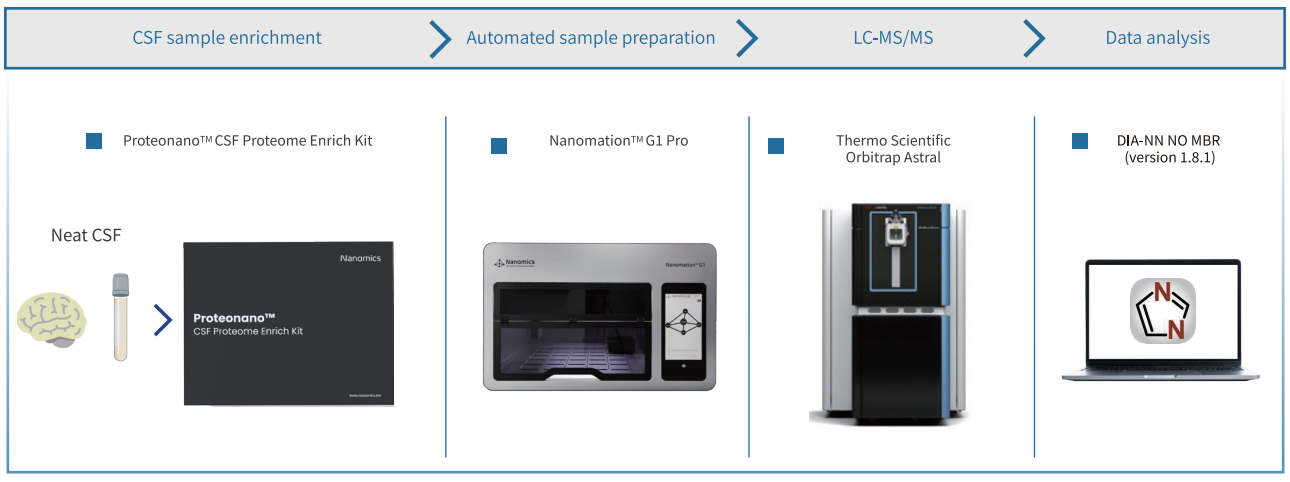

Figure 1: Proteonano™ ultraplex proteomics platform workflow

categorized into targeted and untargeted methods. Untargeted quantitative proteomics analyzes all proteins in a sample without focusing on specific targets, providing a comprehensive view of protein composition.

Enrichment using surface-engineered superparamagnetic nanoparticles has recently become a novel method for targeting low-abundance prot eins[5]. In this study, we will utilize Nanomics Biotech's Proteonano™ CSF Kit and Proteonano™ Ultraplex Proteomics Platform (Pro Suite) (www.nanomics.bio) to analyze cerebrospinal fluid samples and develop a standardized protocol for cerebrospinal fluid proteomics analysis.

Results and Discussion

Results of protein groups identified

The Proteonano™ CSF Kit is designed to enrich proteins in CSF samples. When paired with high-throughput mass spectrometry, it enables high-depth and multiplexed CSF protein analysis, aiding in the discovery of protein biomarkers linked to neurological disorders.

We tested CSF samples with loading amount of 100 ng, 300 ng, and 500 ng using the Orbitra Astral, as detailed in Table 1.

Kit	Sample	Sample loading amount (ng)	Gradient (min)
Proteonano™ CSF Kit	human cerebrospinal fluid	100 300 500	13

Table 1: Experimental parameters for different CSF sample loading amount

Figure 2 shows the number of proteins identified (PGs) at different sample volumes. As the sample volume increases, the number of identified proteins also increases. Even with a minimum sample volume of 100 ng, we were able to identify 1,669 proteins.

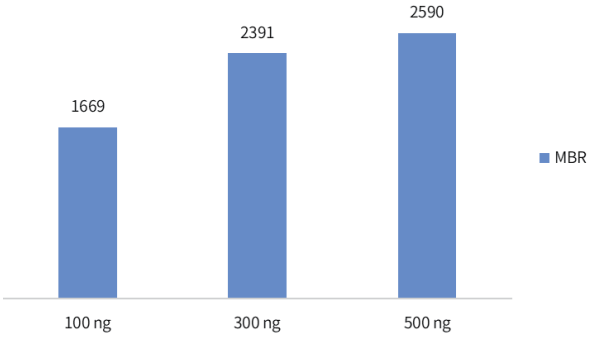

Figure 2: Comparison of different CSF sample loading amount

Dynamic range

With sample volumes of 100 ng, 300 ng, and 500 ng using the Orbitrap Astral, we identified a total of 3,114 proteins. Out of these, 2,395 proteins matched entries in the HPPP database, which includes 4,608 proteins. Additionally, concentration information was available for 2,317 of the identified proteins, offering valuable insights into protein levels in CSF samples.

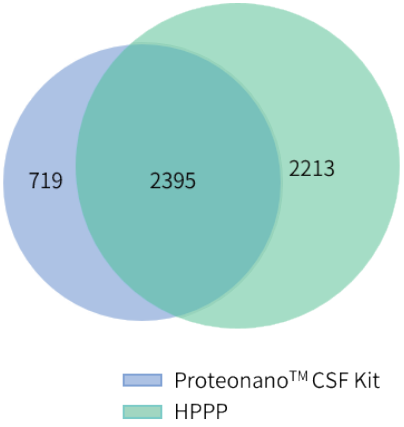

Figure 3: Compare with HPPP database

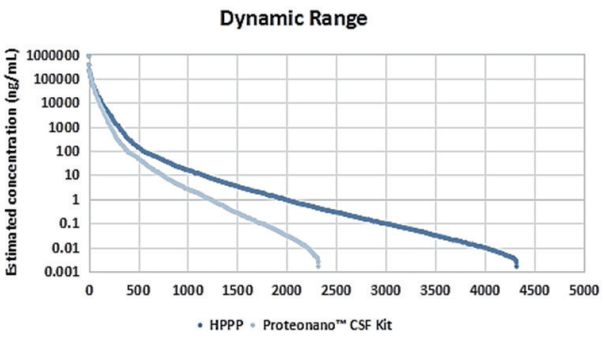

Figure 4: Proteins dynamic range in CSF sample

Conclusion

In this study, we combined the Proteonano™ CSF Proteome Kit and Proteonano™ Ultraplex Proteomics Platform (Pro-suite) from Nanomics Biotech with Thermo Fisher' s latest high-resolution Orbitrap Astral mass spectrometer. Using surface-modified, monodisperse magnetic nanoparticles (S3MNPs), we enriched low-abundance proteins in cerebrospinal fluid (CSF) samples, and performed standardized preparation, protein identification, and analysis. The key findings are:

The volume of CSF sample directly correlates with the number of proteins identified. For instance, a minimum sample volume of 100 ng yielded 1,669 proteins.

We identified a total of 3,114 PGs, including 2,395 PGs that matched entries in the HPPP database and 719 novel PGs.

The analysis covered over 9 orders of magnitude in the CSF proteome.

Overall, the use of the Proteonano™ CSF Proteome Kit for standardized sample preparation, combined with advanced mass spectrometry, enables high-depth detection of low-volume CSF proteomics samples.

Materials and Methods

Protein enrichment reagents

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

LC-MS instruments

- Orbitrap Astral (Thermo Fisher Scientific, Waltham, MA, USA)
- Vanquish Neo (Thermo Fisher Scientific, Waltham, MA, USA)

Data analysis software

- DIA-NN NO MBR (version 1.8.1)

Reference

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