PREOMICS

Overcoming the dynamic range difficulties in CSF samples through a novel enrichment step for biomarker discovery

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SPOTLIGHT

- Fast, simple-to-use and optimized protocol for the preparation of complex samples like cerebrospinal fluid , in high-throughput proteomic profiling approach for biomarker discovery
- ENRICH-iST tackles the challenge of a high diluted protein content and a high dynamic abundance range, which is typical for CSF
- Streamlined solution to reduce the high dynamic range for greater proteomic depth
- Deeper proteome coverage with preserved biological information for discovering additional potential biomarkers

MATERIALS & METHODS

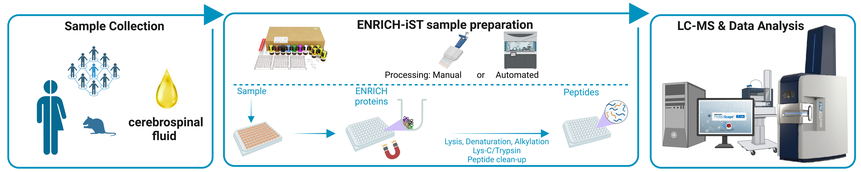
Source:
Cerebrospinal fluid samples from rat.

Sample preparation:
10 µL of CSF followed by iST-BCT protocol (PreOmics) for neat CSF and 20-200 µL of CSF followed by ENRICH-iST protocol.

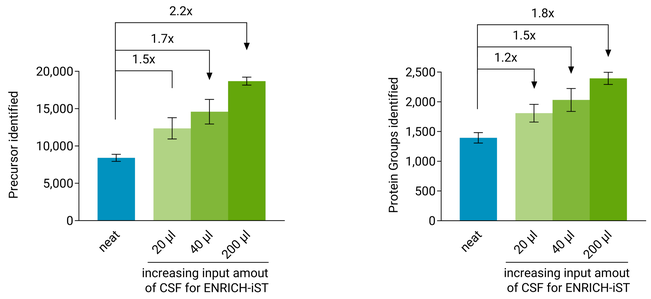
LC-MS analysis:
NanoELUTE2 (24 SPD method) flow rate of 300 nl/min on a reversed-phase C18, IonOpticks Aurora column (25 cm x 75 µm, 1.7 µm) coupled to a timsTOF HT in dia-PASEF mode. Sample load was adjusted to 300 ng load on column.

Data processing:
Burker ProteoScape™ was used for real-time processing of each single MS-run. Afterwards post processing was performed assigning single runs to sub-goups of replicates without match -between-runs.

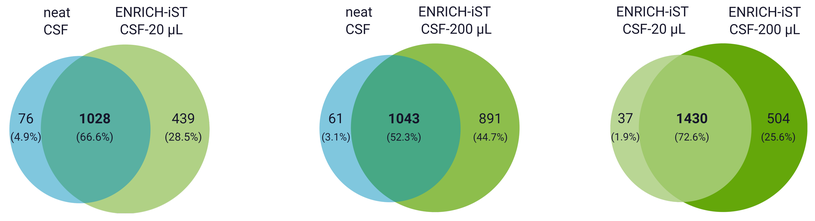
RESULTS



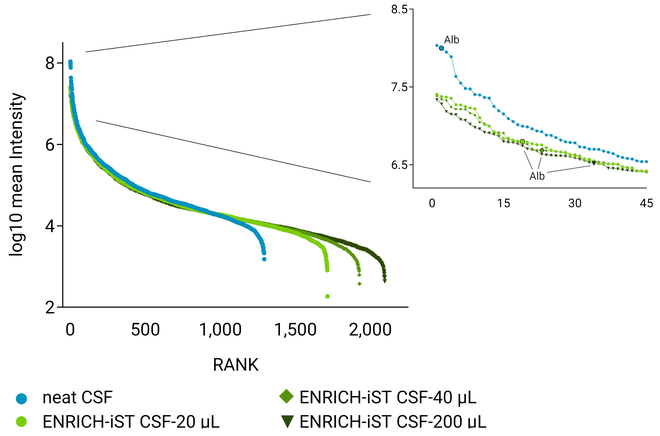
Workflow overview for CSF preparation with iST-BCT and ENRICH-iST kits. Cerebrospinal fluid (CSF) samples from human speciments or animals were prepared with the iST-BCT (neat CSF) and ENRICH-iST kits followed by LC-MS analysis. All data were processed with Bruker ProteoScape (BPS) using timsDIA-NN in library free mode.



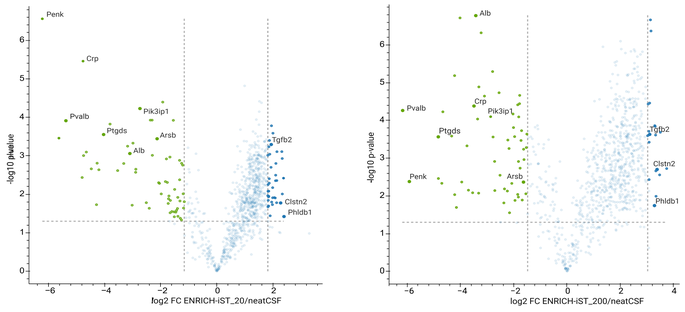
Increased protein identifications by ENRICH-iST. Using the ENRICH-iST approach, precursor and protein group identifications increased. Depending on the starting material, precursor level increase up to a factor of 2.2-fold.On Protein level, 1.8-fold more identified protein groups could be obtained against neat CSF.



Additinal identified proteins in ENRICH-iST prepared samples. Comparing the IDs found in the neat and ENRICH-iST workflows, almost all proteins were detected by ENRICH-iST. Further identifications were possible with a higher starting amount of CSF.



Dynamic range compression by ENRICH-iST. By increasing the amount of starting material, a deeper proteome coverage could be achieved. The ENRICH technology leads to a concentration reduction of high-abundance proteins, such as albumin, resulting in the enrichment of proteins with lower abundance.



Preserved information. The Volcano plot comparing neat CSF and two different ENRICH-iST preparations (20 µL and 200 µL) shows the differently regulated proteins detected in both conditions (neat and ENRICH-iST). In both comparisons, proteins with high abundance were not completely depleted and are still detectable and quantifiable.

KEY TAKEAWAYS

- **ENRICH-iST**: A new pipeline for in-depth, high-throughput plasma proteome profiling is now applicable for cerebrospinal fluid.
- **Enhanced Analysis of Low-Abundance Proteins:** The technology improves the detection of low-abundance proteins, enabling deeper proteome coverage up to 1.8 fold increase and can also deal with low concentrated samples.
- **Drive Biomarker Discovery:** ENRICH-iST is highly effective for discovering new protein biomarkers of cerebrospinal fluid proteomic studies.
- Data processing using ProteoScape™ (timsDIA-NN algorithm) enables real-time analysis of timsTOF MS-runs, resulting in high accurate data for further downstream analysis.

This antibody-free workflow is not species-specific. It can be applied to a variety of mammalian species, which makes it particularly suitable for preclinical studies.

More Information

Conflict of Interest Disclosure:
K.Limm, Z.Demianova, K.Hartinger, and N.A. Kulak are employed by PreOmics GmbH

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