Unique high-throughput ENRICH-iST workflow for deeper plasma proteome coverage enables discovery of novel biomarkers

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SPOTLIGHT

- Fast, easy-to-use, and standardized protocol for preparing blood-derived samples
- High-throughput proteome profiling approach for enhanced biomarker discovery
- Streamlined solution to reduce the high dynamic range in plasma for greater proteomic depth
- Deeper proteome coverage with preserved biological information for discovering additional potential biomarkers

MATERIALS & METHODS

Source: Human plasma samples from a clinical cohort of non-small cell lung cancer (NSCLC) patients (n=9 for neat, n=11 for ENRICH-iST) and healthy donors (n=9 for neat, n=12 for ENRICH-iST).

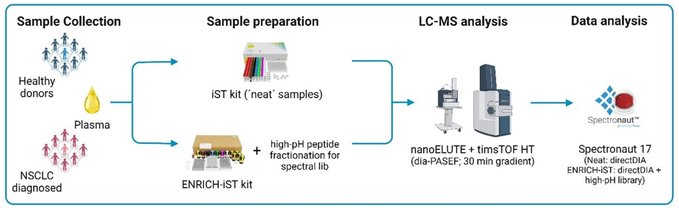
Sample preparation: For neat samples, 2 µL of plasma were processed with the iST protocol. For ENRICH samples, 10 µL of plasma were processed with the ENRICH-iST workflow.

Spectral library: 2x8 high-pH RP separated ENRICH-iST peptide fractions (21,357 precursor spectra 2,200 proteins).

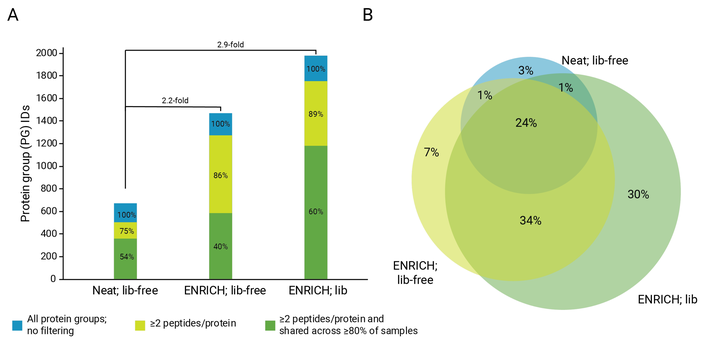
LC-MS analysis: nanoELUTE (30-min gradient) with a PepSep Xtreme column coupled to a timsTOF HT in dia-PASEF mode.

Data processing: Spectronaut 17 using directDIA. ENRICH samples were additionally searched against the spectral library.

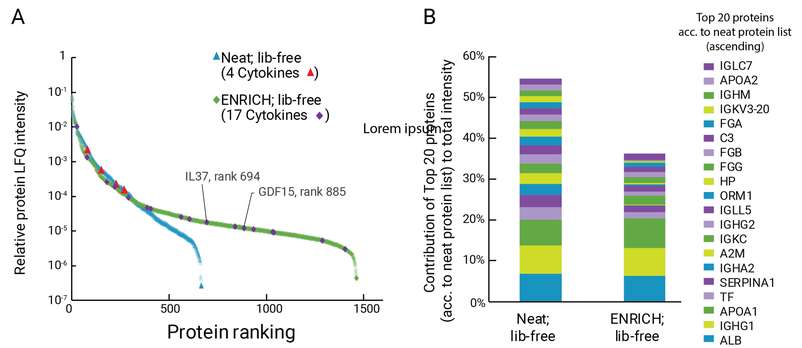
RESULTS



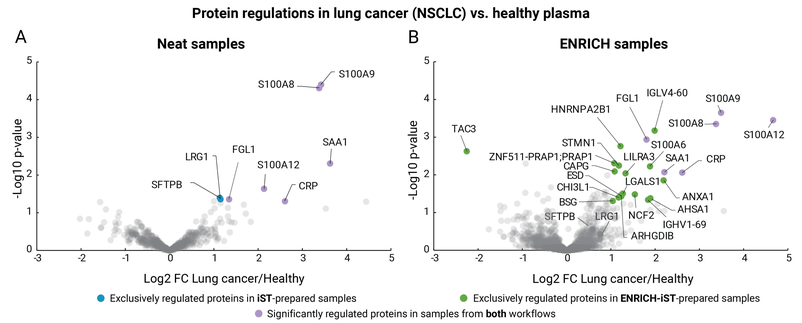
Overview of the cancer study comparing the iST and ENRICH-iST kits. Plasma samples from NSCLC patients and healthy donors were processed with the iST (‘neat’) and ENRICH-iST kits followed by LC-MS analysis. For ENRICH samples, a spectral library was generated using high-pH RP separated ENRICH peptide fractions. All data were processed with Spectronaut using directDIA (´lib-free´). ENRICH samples were additionally searched against the spectral library (´lib´).



Increased protein identifications by ENRICH-iST. A) and B) Protein group identifications increased by 2.2-fold using the ENRICH-iST workflow, with almost no data loss compared to neat samples. The proteome depth could be further enhanced by using library-based data analysis, resulting in an 2.9-fold increase compared to neat samples and superior data quality.



Enhanced proteome coverage by dynamic range compression. A) The ENRICH technology provides deep proteome coverage and enables the analysis of previously undetected low-abundance proteins (such as cytokines), facilitating the discovery of novel biomarkers. B) Effective dynamic range compression is achieved by enriching low-abundance poteins and simultanously reducing the concentration of high-abundance proteins while retaining their information (no depletion).



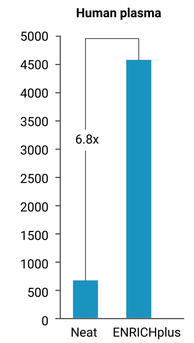
Additional potential biomarkers discovered. Volcano plot analysis showing differences between NSCLC and healthy plasma processed with A) iST or B) ENRICH-iST, revealing potential lung cancer biomarkers. For the iST workflow, eight proteins were identified to be significantly upregulated in NSCLC samples. The ENRICH-iST workflow revealed a similar regulation for six of these proteins and enabled the additional discovery of 16 significantly regulated proteins as potential biomarkers.

KEY TAKEAWAYS

- **Improved protein identification:** ENRICH samples shows a 2.2-fold increase in protein identifications over neat plasma and can be further enhanced to a 2.9-fold increase with spectral library.
- **Enhanced proteome coverage:** The technology improves the detection of low-abundance proteins by efficient dynamic range compression.
- **Extensive potential in biomarker discovery:** Using ENRICH-iST, 16 additional significantly regulated proteins were identified in NSCLC patients.

Overall, ENRICH-iST with state-of-the-art LC-MS analysis and library-based data processing has proven to be a powerful platform for biomarker discovery.

PREVIEW: ENRICHplus



Novel ENRICHplus technology for deepest plasma proteome coverage with up to 7-fold increase in protein IDs compared to neat plasma.

Visit our booth (308) to learn more.

Conflict of Interest Disclosure
M. Abreha is employed by PreOmics Inc K. Limm, Z. Hu, K. Hartinger and N.A. Kulak are employed by PreOmics GmbH A. Schmidt is employed by Bruker Daltonics GmbH & Co. KG S. Mueller is employed by Biognosys AG

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