

## Evaluation of Cell-Free DNA blood plasma for unbiased, deep, and rapid proteomics analysis enabling large-scale studies

### Mass Spectrometry

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While the human genome's protein-coding regions are well known, proteome complexity and composition largely remain elusive. The proteome is dynamic, far more complex, and possesses a diverse structure, composition, and higher number of variants than the genome or transcriptome. However, scaled, deep, and unbiased proteomics studies have been impractical due to the complex workflows required to assess easily accessible samples (e.g., blood plasma).

We have previously described Proteograph™, a novel platform that leverages nanoparticles' nano-bio interactions for deep and unbiased proteomic sampling at scale. Using K2-EDTA (EDTA) plasma samples, we demonstrated the utility of Proteograph in a cohort of early-stage non-small cell lung cancer (NSCLC) subjects and controls. Given the broad adoption of optimized cell-free DNA tubes (BCT) in the field of liquid biopsy, Here, we sought to evaluate plasma samples collected in BCT vs. EDTA blood tubes for unbiased, deep, and rapid proteomics and potentially proteogenomics studies.

Paired blood samples were collected in BCT (STRECK™) and EDTA tubes from 20 subjects of healthy controls and NSCLC patients. Plasma samples were analyzed using only one representative nanoparticle (NP) out of the five included in the Proteograph Product Suite. The digested and purified tryptic peptides were analyzed by nano-LC-MS/MS with a Thermo Scientific UltiMate 3000 RSLCnano system interfaced to an Orbitrap Fusion Lumos Tribrid Mass Spectrometer (Thermo Fisher Scientific) operating in data-dependent mode. MS data were processed using MaxQuant (1% peptide and protein FDR). Comparing the detected peptides and protein groups between collection types, including protein overlap and correlation, precision, and digestion efficiency was evaluated to characterize the similarities and differences of the sample collection tubes.

BCT tubes can be successfully used with standard workflow in Proteograph Product Suite, enabling unbiased, deep, and rapid proteomics studies and potentially multi-omics analysis from commonly obtained Cell-FreeDNA collection tubes.