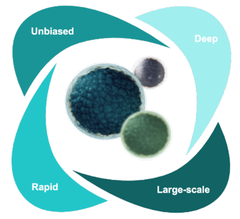
# Human biofluids analysis using a scalable, deep, unbiased, automated, nanoparticle- based proteomics platform

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## The Proteograph™ Product Suite enables rapid sample preparation for reproducible, deep biofluid proteomic analysis

## Introduction

Biospecimen proteomics including characterization of non-blood biofluids like urine are non-invasive and longitudinal sampling source, and other biofluids like cerebral spinal fluid (CSF), and cell line conditioned media (CM) have the potential to reveal new insights to human health and disease. While recent advances in sample collection and mass spectrometry have deepened our understanding of the proteomes for these samples, the field is plagued by non-standardized sample preparation and analytically complex workflows to characterize proteomes at acceptable depth and throughput required for large cohort studies. In this work, we evaluated the utility of the Proteograph™ XT workflow, a standardized, automated multi-nanoparticle-based deep plasma proteomics approach, to interrogate a variety of conditions and sample types spanning CSF and CM.

### Proteograph Product Suite

Seer core technology provides unbiased, deep, and rapid proteomics at scale


**From biospecimen sample to peptides, ready for analysis on most LC-MS instruments with a variety of proteomics methods including label-free and TMT workflows**

## Methods

### Sample Preparation / Data Acquisition

To evaluate the performance of the Proteograph XT workflow, biospecimens were sourced from commercial biobanks to create representative panels of healthy and diseased samples. For human CSF, we profiled samples from normal donors and donors with Parkinson's disease (PD), Amyotropic Lateral Sclerosis (ALS), and healthy individuals. To assess *in vitro* secretome models, CM samples from iPSC, breast, colon, cervical, and prostate cancer cell lines were analyzed.

Samples were processed directly using the Proteograph XT Assay Kit (Seer Inc.), and in parallel with conventional neat sample digestion as a control. Tryptic peptides were analyzed by DIA LC-MS analysis using an Orbitrap™ Exploris™ 480 mass spectrometer, and data processing was performed using Proteograph Analysis Suite.

### Data Acquisition / Analysis

For Data-Independent Acquisition (DIA), 200 – 400 ng of peptides in 4 µL were reconstituted in a solution of 0.1% formic acid (FA) and 3% acetonitrile (ACN) spiked with 5 fmol/µL PepCalMix from SCIEX for constant mass MS injection between samples regardless of starting volume. DIA data was processed using Proteograph Analysis Suite 2.1. Raw MS data was processed using the DIA-NN search engine (version 1.8.1) in library-free mode searching MS/MS spectra against an *in silico* generated spectral library of human protein entries (UP000005640_9606) or human + bovine (UP000009136_9913) *in silico* generated spectral library (for conditioned media samples).

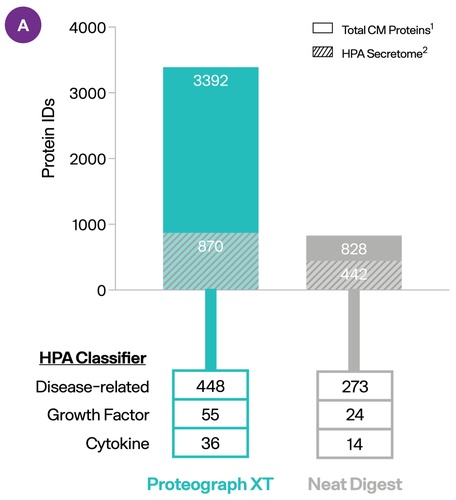

**Figure 1. Proteograph XT Assay Performance Comparison and Quantitative Expression Profiles.**

Protein identifications for CM samples **(A)** and CSF samples **(B)** using Proteograph XT Assay with standard assay protocol compared to baseline direct digest (DD), with error bars denoting standard deviation (n= 3); teal bars= Proteograph, grey bars = direct digest. **(C)** Principal component analysis (PCA) of complete panel of biofluids indicating high reproducibility and distinct profiles between biofluid types.

## Proteograph XT Assay enables detection of quantitative expression differences and specific functional pathway enrichments for variety of biofluids


**Figure 2. Evaluation of Quantitative Reproducibility for Conditioned Media Analysis.**

**(A)** Correlation analysis of triplicate samples from CM secretomes representing iPSC, breast, colon, cervical, and prostate cancer cell lines (Pearson's correlation coefficient based on protein intensity shown). **(B)** Exemplars of inter-replicate correlation within a cell line sample, BT549 + FBS condition replicates shown (Pearson's correlation analysis for common proteins between replicates).




B

**Representative cytokines detected in serum-containing CM**

| | | | | | |
|---|---|---|---|---|---|
| AIMP1 | BMP1 | BMP4 | BMP6 | C1QTNF4 | CCL14 |
| CCL2 | CCL3 | CCL5 | CSF1 | CXCL1 | CXCL10 |
| CXCL12 | CXCL2 | CXCL3 | CXCL5 | CXCL6 | CXCL8 |
| GDF11 | GDF15 | GDF2 | GPI | GREM1 | GRN |
| IL17C | IL36A | IL6 | LEFTY1 | LEFTY2 | MIF |
| MSTN | NAMPT | PF4 | PPBP | SPP1 | TAFA5 |

Unique to Proteograph XT, not in neat digest results

**Figure 3. Annotation of Disease-related Proteins, Growth Factors and Cytokines Detected with Proteograph XT.**

**(A)** Protein IDs from the +FBS conditions across all cell lines in this study were queried against the Human Protein Atlas (HPA) annotated secretome for entries with disease-related association and keywords 'Growth Factor' or 'Cytokine'. **(B)** Representative cytokines detected in serum-containing CM are shown.

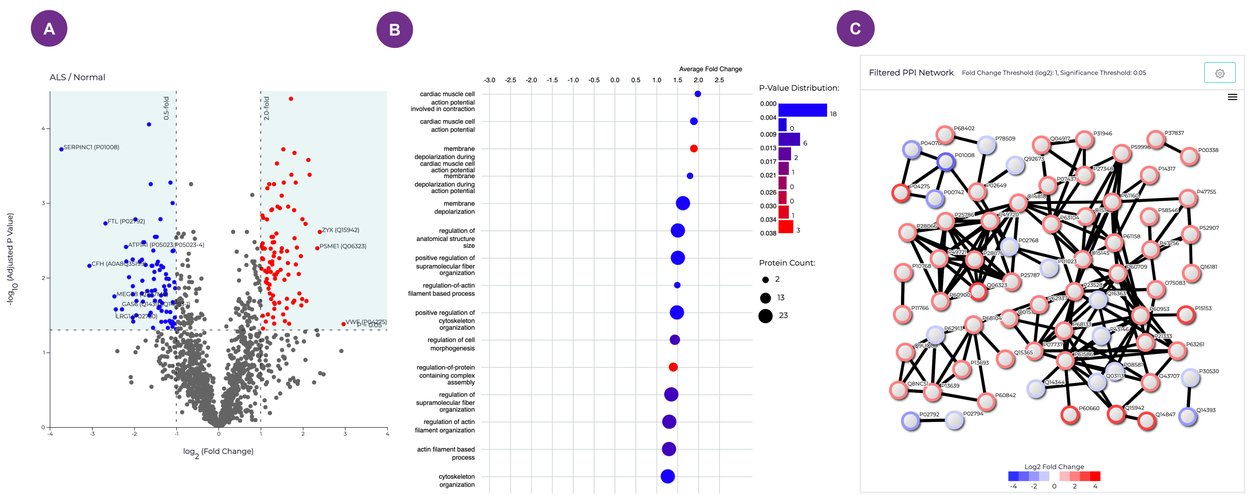



**Figure 4. Differential Expression Analysis of CSF Proteomes from Amyotropic Lateral Sclerosis (ALS) and Normal Donors. (A)** Example of differential expression analysis showing significantly regulated protein groups detected between CSF biospecimens representing Amyotropic Lateral Sclerosis (ALS) compared to normal donors (>2.0 fold and adjusted p value <= 0.05). **(B)** Gene Ontology enrichment analysis of significantly regulated proteins in CSF samples from ALS patients compared to normal donors. **(C)** STRING protein-interactome plot of significantly regulated proteins from CSF samples from ALS patients compared to normal donors.

## Conclusions

- The standard Proteograph XT Assay protocol was used with a range of biofluids including serum/plasma, CM, and CSF samples and required no additional pre-processing steps.
- Proteograph workflow improves proteome coverage for CM and CSF compared to conventional neat digest protocols (1.5x – 12x improvement), and ~2x more proteins classified as growth factors or cytokines.
- Proteograph XT was used with conditioned media samples grown in serum-containing conditions, obviating the need for FBS starvation and additional sample manipulations.
- Deep proteomic coverage, high assay reproducibility, and proteome pathway insights are possible with the standard Proteograph XT Assay protocol for CSF and conditioned media samples.

Publications



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