TP 744

# High Throughput Abundant Protein Depletion for Plasma Proteomics

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## Abstract

Biological fluids like plasma are widely used for proteomics-based disease biomarker discovery. However, its analysis is challenging due to the complexity and wide dynamic range of the proteome, ranging over 10 orders of magnitude. The high abundant proteins like albumin and immunoglobulins (IgGs) constitute about 90% of the plasma, and their presence masks the identification of the low-abundant proteins in the sample. Recently, we developed an abundant protein depletion resin that uses highly specific antibodies to deplete these abundant proteins. Currently, we have developed a new 96-well filter plate format to support high throughput plasma proteomics. This format showed nearly identical performance compared to our spin column format and depletes 90-95% of these abundant proteins with high reproducibility.

## Introduction

The wide dynamic range in the protein concentration of plasma and serum remains a major challenge as the high abundant proteins interfere with the identification and quantitation of the low abundant proteins. These highly abundant proteins including albumin and IgGs constitute about 90% of the plasma protein content. Several workflows have been developed around depleting these abundant proteins to enable deeper analysis of the proteome to identify low-abundant proteins of interest. These workflows are laborious with multi-step protocols which greatly increases hands-on-time while processing multiple sample types. To address these issues, we developed a 96-well plate format of the depletion resin to support higher sample throughput. This format can deplete 96 samples at a time with a depletion efficiency of ≥ 90% and showed similar performance to our spin column format in terms of protein/peptide identifications and Top14 abundant proteins depletion in the plasma samples.

We have also evaluated our format with different biological fluids, such as CSF. In addition, our depletion resin in 96 well formats combined with our optimized EasyPep chemistry can process these samples in 7-8 hours with high reproducibility and <5% CVs. These two combined can significantly eliminate the hands-on challenges while handling a large number of samples.

## Materials and methods

### Sample Preparation

Several sample types, including Plasma, serum and CSF were depleted using our Thermo Scientific™ High-Select™ Top14 Abundant Protein Depletion Resin in a 96-well format and processed using our standardized Thermo Scientific™ EasyPep™ 96 sample preparation procedures. A label-free data-independent acquisition (DIA) strategy was used to quantify differentially expressed proteins for the cancer vs. normal plasma samples. Peptide yields were assessed using Thermo Scientific™ Pierce™ Quantitative Colorimetric or Fluorometric Peptide Assay.

### LC-MS Analysis

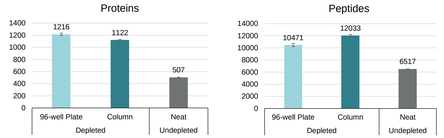
Samples were separated using a Thermo Scientific™ Vanquish™ Neo UPLC system using a 50 cm C18 Thermo Scientific™ EASY-Spray™ PepMap™ Neo column with an acetonitrile gradient from 3% to 28% over 85 min, 28% to 45% over 30 min, at a flow rate of 300nL/min on a Thermo Scientific™ Orbitrap™ Eclipse™ Tribrid™ Mass spectrometer or Thermo Scientific™ Orbitrap™ Exploris™ 480 Mass spectrometer or Thermo Scientific™ Orbitrap™ Astral™ Mass spectrometer.

### Data Analysis

LC-MS data were analyzed using the SEQUEST® HT search engine in Thermo Scientific™ Proteome Discoverer™ 3.0 software using static carbamidomethyl (C), dynamic oxidation (M), and deamidation (N,Q) modifications. Data were searched against the UniProt human protein database and results were filtered using a 1% protein FDR threshold. DIA data was searched using CHIMERYS™ search algorithm in Thermo Scientific™ Proteome Discoverer™ 3.1 software.

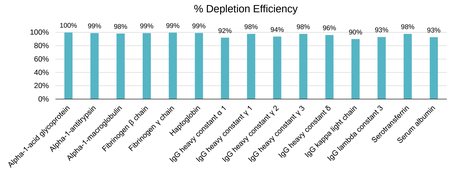
## Results

**Figure 1: Assessing performance of the depletion resin in plate format compared to spin column format**



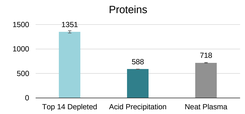

10 µl of human plasma was depleted in triplicates using our antibody-based depletion resin in a 96-well plate format and a column format. The depleted plasma samples were processed with EasyPep technology. Protein digest (200 ng) was analyzed using Thermo Scientific™ Orbitrap™ Astral™ Mass spectrometer. The results demonstrate that the number of proteins and peptides identified with the 96-well format is nearly identical to the spin column format.

**Figure 2: Depletion efficiency of the Top14 Abundant proteins from human plasma using the 96-well plate format**



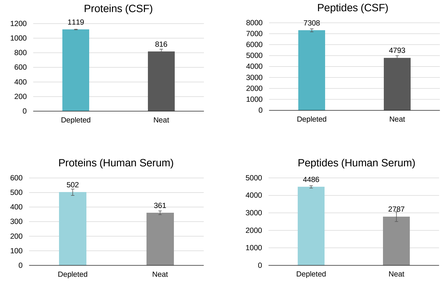

Human Plasma was depleted using our antibody-based depletion resin in a 96-well plate format using our optimized protocol. The depleted plasma samples were processed with EasyPep technology. Protein digest (250 ng) was analyzed by LC-MS. The depletion efficiency of the depleted proteins was calculated based on the abundances in the depleted samples compared to the neat sample. The results demonstrate that the optimized protocol depletes the Top 14 Abundant proteins with a depletion efficiency of ≥90%.

**Figure 3: Better performance than conventional protocol (acid precipitation)**



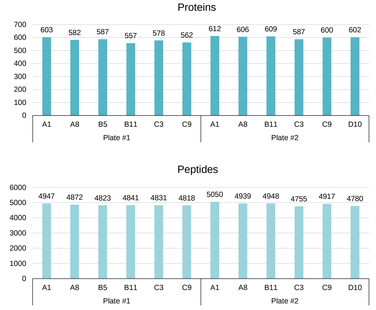

Human plasma was depleted using our antibody-based depletion resin in a 96-well plate format using our optimized protocol followed by processing with EasyPep technology. Plasma samples were also prepared using Perchloric acid precipitation protocol. Protein digest (250 ng) was analyzed using Thermo Scientific™ Orbitrap™ Astral™ Mass spectrometer. The results demonstrate that Top 14 depleted plasma samples yield higher protein IDs compared to acid-precipitated samples.

**Figure 4: Depletion using different biological fluids**



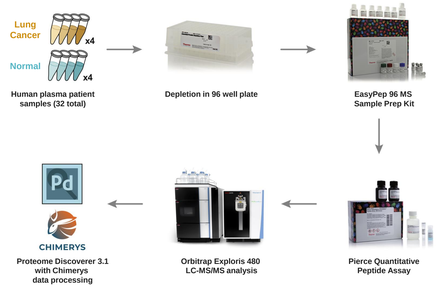

50µl of human CSF (Parkinson's disease) and 10µl of human serum was depleted using our antibody-based depletion resin in a 96-well plate format. The depleted plasma samples were processed with EasyPep technology. Protein digest (250 ng) was analyzed by LC-MS. The results demonstrate that our Top14 depletion resin can deplete other human biofluids such as CSF and human serum with ≤ 5 CVs% and improve the protein IDs by 37% and 39% for CSF and serum, respectively.

**Figure 5: Assessing workflow reproducibility**



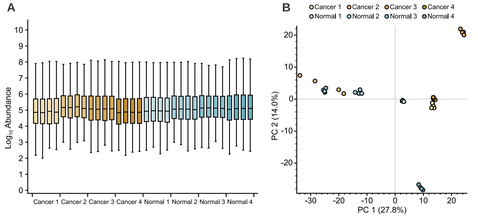

10 µl of human plasma was depleted using our Top 14 Abundant Protein depletion resin in a 96-well plate format using our optimized protocol on two plates followed by processing with EasyPep 96 MS kit. Eight samples out of each plate were randomly selected and ~250 ng was analyzed by LC-MS/MS. The results show that our workflow is reproducible between the plates and yielded similar protein and peptide IDs with CVs < 10%.

**Figure 6: Schematic of the Label-free quantitation experiment using a data-independent acquisition (DIA) with human plasma normal and non-small cell lung cancer samples**




Human plasma samples were collected from 4 normal and 4 lung cancer patients from different race, age, etc. generating a sample size of 32 samples. 10 µl of human plasma (normal and cancer) samples were depleted using our antibody-based depletion resin in a 96-well plate format followed up by processing with EasyPep technology. Human Plasma digest (~250ng) was analyzed using DIA on a mass spectrometer.

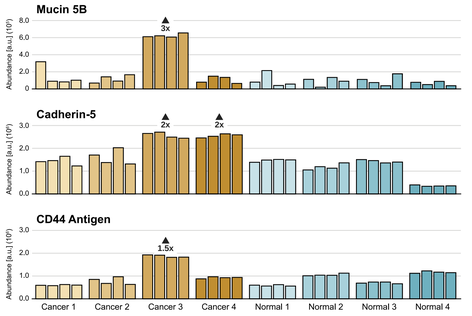
**Figure 7: Label-free quantitation of human normal vs cancer plasma samples**




A. Box-Whisker plot showing label free quantification. Overall, relative quantitation was observed to be the same across all 32 samples.

B. Around 1500 unique proteins and 10,000 unique peptides were identified. Principal component analysis grouped the biological replicates based on similar protein/peptide abundance.

**Figure 8: Example of targeted disease biomarker from human plasma normal and cancer samples**




A. Alteration in Mucin 5B contribute to mucus dysfunction in asthma and lung cancer. Mucin 5B levels showed a 3-fold increase in one of the cancer samples compared to normal plasma samples.

B. Cadherin-5 (VE-cadherin) plays a crucial role in postnatal angiogenesis and is a potential target for antiangiogenic treatment. Cadherin 5 levels showed a 2-fold increase in two of the cancer samples compared to normal plasma samples.

C. CD44 Antigen showed a 1.5-fold increase in one of the cancer samples compared to normal plasma samples.

We were also able to identify other targeted disease biomarkers such as cardiovascular and oncology biomarkers along with the lung cancer biomarkers from the patient plasma samples. But there is a need for a larger sample cohort size of the plasma patient samples to accurately quantify the fold difference in the expression of these proteins.

## Conclusions

- Our depletion resin in a 96-well plate format enables the depletion of Top 14 abundant proteins from the biological fluids with a greater depletion efficiency of ≥ 90% with high reproducibility and <5% CVs for high throughput plasma proteomics.
- Our depletion resin can deplete the abundant proteins from different biological fluids, such as plasma, serum, and CSF.

## Trademarks/licensing

For Research Use Only. Not for use in diagnostic procedures.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