

Absolute Quantification of the Lower Abundance Proteome Through Immunoaffinity Depletion of the Twenty Most Abundant Proteins in Human Serum



Jeffrey J. Porter, Justin Wildsmith, Christopher D. Melm, Mark D. Schuchard,
Kevin M. Ray, Dian Er Chen, and Graham B.I. Scott

Sigma-Aldrich Biotechnology
P.O. Box 14508
St. Louis, MO 63178

Abstract

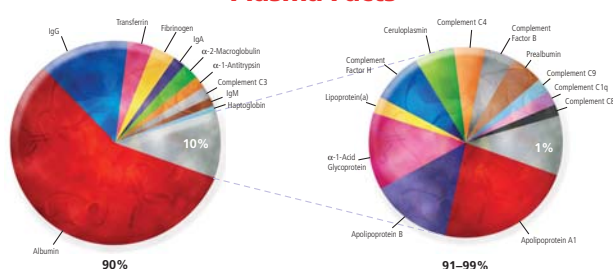
Plasma proteomics holds great promise for the future of biomarker discovery, as well as in vitro diagnostics. Although plasma is readily accessible for analysis, the study of the plasma proteome is fundamentally limited by its vast dynamic range (10 orders of magnitude). Low-abundance proteins, carrying great diagnostic potential, are often obscured by the presence of high-abundance serum proteins. In order to address this problem, Sigma-Aldrich has developed the ProteoPrep® 20, an antibody-based resin capable of depleting 97–98% of the total protein from plasma. Depletion of these high-abundance proteins allows for visualization of proteins co-eluting with, and masked by, the high-abundance proteins and peptides, using LC-MS methods.

Absolute Quantification (Protein-AQUA™) is a targeted quantitative proteomics technique that exhibits robust efficacy and is being increasingly utilized for a wide variety of quantitative proteomics studies. By applying the ProteoPrep 20 for depletion of the most abundant proteins, we have enabled the identification and absolute quantification of numerous lower-abundance proteins from human serum. We believe this may potentially provide a multiplexed targeted approach to the discovery of proteins holding biological or diagnostic significance.

Introduction

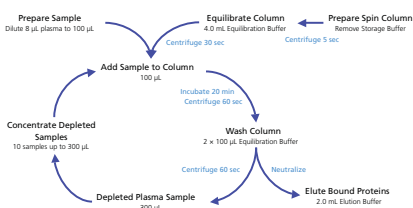
The study of the human plasma proteome is an area of great interest, especially for the pharmaceutical potential of identifying disease biomarkers. Many proteins of pharmaceutical interest appear at low concentrations in the plasma and are, therefore, difficult to detect. Identification of potential biomarkers is especially difficult due to the presence of higher-abundance proteins. Depletion of these high-abundance proteins allows for visualization of proteins co-eluting with, and masked by, the high-abundance proteins and peptides, using LC-MS methods. By removing the high-abundance proteins, higher loads of lower-abundance plasma proteins can then be separated on reverse phase columns, allowing detection and quantification of low copy number proteins. The ProteoPrep 20 Plasma Immunodepletion Kit has been developed for removal of 20 high-abundance proteins from 8 μ L of plasma. Depletion of these 20 high-abundance proteins removes greater than 97% of the proteins in plasma and permits analysis of 20- to 50-fold more of each individual protein for improved visualization of lower copy number proteins. By applying the ProteoPrep 20 for depletion of the 20 most abundant proteins, we have enabled the identification and absolute quantification of numerous lower-abundance proteins from human serum.

Plasma Facts



- The 10 most abundant proteins represent approximately 90% of the total protein mass in human plasma.
- The 22 most abundant proteins are said to represent approximately 99% of the total protein mass in human plasma.
- The ProteoPrep 20 plasma immunodepletion column removes the 20 high-abundance plasma/serum proteins listed below. These 20 proteins represent approximately 97% of the total human plasma protein mass.

Albumin	α -2-Macroglobulin	Apolipoprotein A1	Complement C4
IgGs	IgMs	Apolipoprotein A2	Complement C1q
Transferrin	α -1-Antitrypsin	Apolipoprotein B	IgDs
Fibrinogen	Complement C3	Acid-1-Glycoprotein	Prealbumin
IgAs	Haptoglobin	Ceruloplasmin	Plasminogen



Methods

High-Abundance Protein Depletion

Twenty high-abundance proteins were depleted from plasma using the ProteoPrep 20 Immunodepletion Kit (PROT20). A large 3.7-mL prototype spin column capable of depleting 100 μ L of human plasma was used. The protein concentration of whole and depleted plasma was determined using Bradford assay (B6916), with BSA as the standard.

Tryptic Digestion

Samples of both whole and depleted plasma were prepared in 100 μ g aliquots. Each sample was diluted to 0.25 mg/mL in ammonium bicarbonate buffer. Acetonitrile was added to a final concentration of 9% (v/v), and each sample was reduced and alkylated using the ProteoPrep Reduction/Alkylation Kit (PROTRA). Proteomics Grade Trypsin (T6567) was added at a concentration of 1% (w/w) and the sample incubated for 3 hours at 37 $^{\circ}$ C. An additional aliquot of trypsin at 1% (w/w) was added and then the sample further incubated at 37 $^{\circ}$ C overnight. The digests were dried to completion in a SpeedVac™.

LC-MS/MS Analysis

An injection of a tryptically digested ProteoPrep 20 depleted plasma sample (prepared as described in Methods) was made using an Agilent capillary 1100 HPLC. The reverse phase separation was performed on a 15 cm \times 2.1 mm Discovery C18 column, using a 3-hour gradient. The mobile phase consisted of formic acidified water and acetonitrile. The separation system was coupled to a Thermo Finnigan LTQ linear ion trap mass spectrometer set to perform tandem MS on the ten most abundant ions in each full scan spectrum. After obtaining tandem MS results on an ion of interest, it was added to an exclusion list to facilitate the interrogation of lower-abundance ions. An LC-SRM method was developed corresponding to the protein targets albumin (ideotypic peptide = FQNALLVR) and gelsolin (ideotypic peptide = GASQAGAPQGR), as outlined in the Results section.

AQUA Analysis

An AQUA Peptide stock solution was prepared by dissolving isotopically labeled versions of the two target peptides (FQNALLVR* and GASQAGAPQGR*, respectively) in 0.1% TFA to a final concentration of 62.5 fmol/ μ L. Each digest sample (whole and depleted plasma) was dissolved in 20 μ L of the AQUA Peptide stock solution. Samples were analyzed using an Agilent capillary 1100 HPLC coupled to a Thermo Finnigan LTQ linear ion trap mass spectrometer. Using an LC-SRM method, the absolute quantity of each peptide (and corresponding protein) was determined.

The Protein-AQUA Method

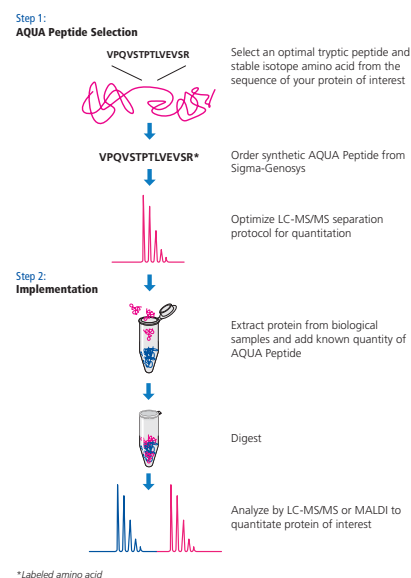


Figure 1: Overview of the Protein-AQUA Method. This method was developed by Dr. Steve Gygi and colleagues at Harvard Medical School [Stemann O., Zou H., Gerber S. A., Gygi S. P., Kirschner M. W.; Dual inhibition of sister chromatid separation at metaphase, Cell 2001, Dec 14, 107: 715-726]. Limited use of this method is permitted under a licensing arrangement with Harvard Medical School.

