

## Western Blotting Application in Analysis of Proteins

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### DESCRIPTION

Plasma proteomics is the high-throughput analysis of plasma biomarkers combining extremely powerful, sensitive, and specific tools. Blood plasma is one of the most commonly used samples in cancer biomarker discovery research, as well as clinical trials for diagnostic and therapeutic purposes. The most common type of biological test performed in clinical practice and laboratory testing is blood tests. They are based on changes in the concentration of different proteins in the blood. However, doing so on a large scale by continuously monitoring all blood proteins (i.e., the blood proteome) provides an accurate picture of protein expression in health and sickness [1]. The plasma proteome, on the other hand, is extremely complex due to its wide dynamic range of protein concentrations and the presence of many proteins. The most advanced technique for plasma protein analysis was the 2D PAGE method, which could isolate up to 289 proteins. Serum albumin, globulins, and complement factors constitute for more than 99 percent of the entire plasma proteome, making it one of the most complex and diverse sub-proteomes in the human body [2]. Blood proteins are emerging as potential biomarkers for many diseases.

Novel sample preparation methods have also evolved to facilitate in the detection of proteins found at extremely low amounts in plasma. Affinity purification is used to acquire a specific protein, and immunochemistry is used to eliminate proteins at high concentrations. Furthermore, the introduction of high-speed Mass Spectrometry (MS) techniques and the advent of LC systems for long-term nanoscale flows have enabled robust workflows in proteomics to deepen analysis. One of the main advantages of plasma proteomics is the easy availability of blood in the peripheral circulation, which allows for simple sample collection. Blood also serves as a buffer for all tissues and cells, transporting compounds that are generated, released, destroyed, or absorbed at various places. There are many disadvantages as well regarding this process. Circulating biomarkers have received greater attention than site-specific blood indicators. However, unless the marker is specific itself, such as troponin T, which is released only by damaged cardiac myocytes, variations in circulating biomarkers could reflect disease or dysregulation of any other organ rather than a specific disorder [3]. Another issue

is the increased signal-to-noise ratio, which lowers predictive value and thus therapeutic relevance. This can only be addressed by employing interventional methods to collect blood samples from a vascular coming from the organ of interest, which is usually not possible. Another issue is that in addition to the huge number of proteins, most of them are known to exist in variety of forms after post-translational modifications. Plasma proteins shift as a result of various metabolic and physiological states and stages. There are also significant changes in the type and number of measured proteins expressed in plasma according on the sample type, namely plasma *vs* tissues or cells. Several advanced analytical approaches are being used to address this, with an emphasis on controlling the complexity of plasma proteins and their dynamic range. By combining plasma proteomics with immune depletion of high- and medium-abundance proteins as well as highly extensive peptide fractionation methods, it is now possible to detect more than 1,000, if not more than 5,000, proteins in plasma [4]. However, MS-based plasma proteomics is incredibly challenging for a variety of reasons, the most prominent among which is the unusually wide dynamic range of protein abundances.

### CONCLUSION

Plasma proteomics has the potential to significantly improve in the prediction and treatment of metabolic and other chronic disorders, as well as the identification of adverse medication effects. The discovery of new biomarkers for illness conditions necessitates the identification of prospective biomarkers through case-control studies or, more recently, longitudinal investigations. This is then confirmed through targeted analysis of a larger number of plasma samples. Finally, the potential markers are clinically evaluated in large interest to assess their sensitivity, specificity, and utility.

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**Received:** 03-Oct-2022, Manuscript No. JPB-22-19719; **Editor assigned:** 06-Oct-2022, PreQC No. JPB-22-19719 (PQ); **Reviewed:** 20-Oct-2022, QC No. JPB-22-19719; **Revised:** 27-Oct-2022, Manuscript No. JPB-22-19719 (R); **Published:** 03-Nov-2022, DOI: 10.35248/0974-276X.22.15.611

**Citation:** Surette M (2022) Western Blotting Application in Analysis of Proteins. *J Proteomics Bioinform.* 15:611

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