Case study | 004412

Thermo Fisher SCIENTIFIC

Plasma proteomics

Absolute quantification of plasma proteins using recombinant protein standards and high-throughput targeted mass spectrometry

Introduction

In modern biomedical research, precision is no longer a luxury; it is the foundation upon which meaningful insights are built. Yet in the field of plasma proteomics, achieving high precision and quantitative accuracy of proteins at scale long remained a major challenge. Variability in sample preparation, inconsistent workflows, limited targeted multiplexing capacity, and an inability to scale have left many promising biomarker candidates stranded in the gap between discovery and clinical implementation.

Recombinant protein standards that undergo identical processing as endogenous proteins offer a strategy to control analytical variability and support absolute protein quantification. In this study, we evaluate the performance of a ProteomEdge™ Quantitative Recombinant Protein Standard (qRePS)-based workflow with the Thermo Scientific™ Stellar™ Mass Spectrometer in a longitudinal plasma study.

The qRePS platform: Engineered for reliability

The centerpiece of ProteomEdge's innovation is qRePS, which introduces a more biologically accurate, reliable, and scalable method for absolute protein quantification than traditional peptide-based or post-digestion spike-ins. Each qRePS is a recombinant protein fragment consisting of a sequence of approximately 100 amino acids developed and optimized for generation of proteotypic peptides for mass spectrometry (MS) analysis. The qRePS is metabolically labeled with heavy isotopes (with >99% isotopic incorporation), then pre-aliquoted and dried into a 96-well plate for easy, highly consistent use with a sample volume equivalent of 1 µL neat blood plasma (Figure 1).

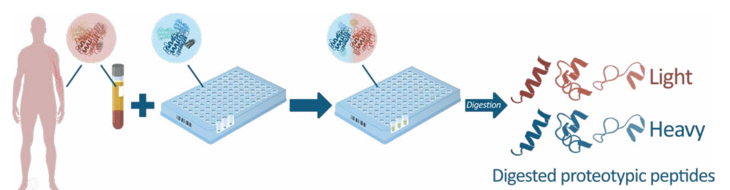

Figure 1. qRePS workflow. In the qRePS workflow, 1 µL of plasma is added directly to the pre-aliquoted and dried heavy-labelled protein-fragment standards contained in each well of the 96-well plate. Introducing the internal standards at the start of sample preparation ensures that subsequent digestion and handling steps affect heavy (qRePS-derived) and light (endogenous) peptides in the same way. A standard bottom-up proteomics workflow is then performed in the well, generating heavy/light peptide pairs that enable accurate LC-MS/MS-based quantification.

thermo scientific

What makes qRePS novel is its ability to deliver true absolute quantification across both focused disease targets and large-scale discovery panels, bringing flexibility and analytical rigor into a single, ready-to-use format.

To understand the impact of this design, it's helpful to look at how qRePS transforms the core elements of quantitative proteomics (Figures 2 and 3). These core capability enhancements are summarized in the table below.

Capability	Why it matters
Identical processing of standards and samples	qRePS undergoes every step of the workflow, from thawing through digestion and cleanup, mirroring the sample precisely and ensuring any variability introduced along the way affects both equally, allowing process-driven bias to be detected, monitored, and corrected.
Detection of discordance between peptide- and protein-level measurements	By generating multiple peptides per protein, qRePS enables researchers to differentiate true biological changes leading to clearer interpretation and more accurate protein quantification.
Multi-peptide quantification	Each qRePS protein produces several heavy-labeled peptides during digestion, creating multiple independent measurements for the same protein. This redundancy reduces noise from peptide-specific effects (ion suppression, digestion efficiency, etc.) and significantly increases confidence in the final absolute values.
Room-temperature stability	Product plates with vacuum-dried qRePS remain stable at ambient temperatures, eliminating cold-chain shipping requirements and preventing freeze-thaw degradation. Labs anywhere in the world can receive plates ready for immediate use with consistent performance.
Multiplexed coverage	qRePS supports panels ranging from carefully selected, clinically relevant disease-specific proteins to broad, high-density discovery sets, enabling measurement of hundreds of plasma proteins in a single workflow. This breadth allows pathway-specific analyses and systems-level profiling using the same standardized technology.
Automation readiness	With pre-aliquoted, workflow-integrated standards in a 96-well plate, qRePS fits directly into automated liquid-handling platforms. High-throughput labs can minimize manual pipetting, reduce operator variability, and scale up with ease.

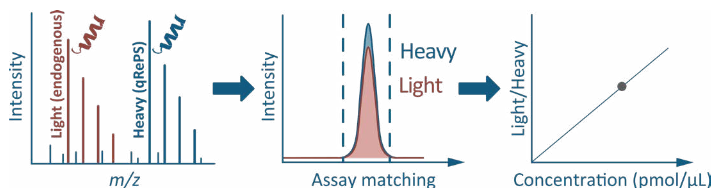

Figure 2. Principle of absolute quantification using qRePS. After LC-MS/MS analysis, the ratio between the heavy (qRePS-derived) and light (endogenous) peptide signals is used to calculate absolute protein concentrations. Because qRePS is supplied at known amounts, the endogenous concentration can be determined directly from the heavy-to-light ratio. This approach supports precise quantification across samples without requiring external calibration curves.

Figure 3. qRePS panels. Each qRePS panel contains the full set of heavy-labelled protein-fragment standards in every well, enabling multiplex, multi-peptide quantification of the targeted proteins. The dried format supports consistent sample handling and facilitates integration into both manual and automated workflows. Stability at ambient temperature also simplifies storage and transport.

Together, these capabilities transform plasma proteomics by eliminating pre-analytical noise, driving unprecedented reproducibility, and delivering the level of precision required for true biomarker discovery and translation (Figure 4). ProteomEdge's expanding suite of panels reflects the complexity of human plasma and the diversity of scientific use cases. Each panel is built to deliver not just protein coverage, but analytical confidence, an essential requirement for longitudinal studies, clinical cohorts, and validation-ready workflows.

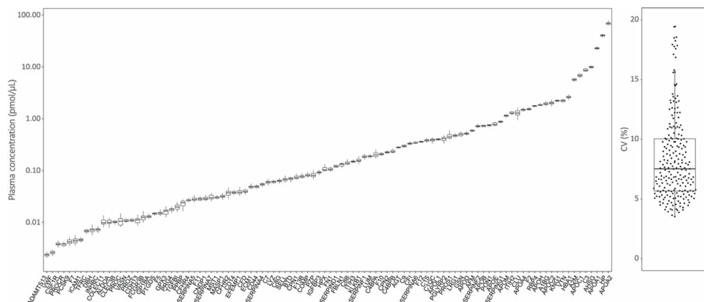

Figure 4. Technical performance across plates and replicates. Using Skyline software for data processing, absolute concentrations were obtained for >100 proteins involved in immune regulation, coagulation, lipid metabolism, and complement pathways. Across 20 biological replicates measured on 4 qRePS plates, the median coefficient of variation (CV) was 7.5%. These results demonstrate consistent performance across plates without the need for batch correction or normalization.

Quantifying the maternal proteome with qRePS for real-world biology

A compelling demonstration of the qRePS platform in action comes from ProteomEdge's longitudinal pregnancy cohort study, which was designed to characterize trimester-specific proteomic shifts with high analytical rigor. In this study, 300 longitudinal plasma samples were collected from 100 individuals, sampled once per trimester. Each sample together with 20 workflow replicates were spiked with the ProteomEdge™ DiscoveryEdge™175 qRePS panel and analyzed using an Evosep One™ system coupled to the Stellar mass spectrometer—a pairing chosen for its quantitative stability, wide dynamic range, and streamlined method setup.

The Stellar mass spectrometer's fast MS/MS acquisition and optimized ion handling enabled a throughput of 40 samples per day (SPD), allowing the ability to run the entire cohort efficiently without compromising spectral quality. The combination of qRePS co-digestion and the Stellar mass spectrometer's robust parallel-reaction monitoring (PRM) and data-independent acquisition (DIA) performance ensured highly reproducible quantification across the plasma matrix, a notoriously challenging sample type due to its broad dynamic range.

This performance reflects the seamless integration of the Stellar mass spectrometer's acquisition intelligence with modern, software-driven method automation. Skyline software's PRM Conductor accelerates targeted method development by automatically selecting optimal peptides and defining acquisition settings from discovery data.

A defining capability of the Stellar mass spectrometer is adaptive retention time (adaptive RT), which continuously adjusts retention-time (RT) windows in real time, preserving the detection of the precise and intact peak despite inevitable RT drift across large-scale studies, column changes, or fast-gradient conditions. By dynamically refining scheduling rather than relying on static RT predictions, adaptive RT reduces manual intervention, improves robustness across extended sample series, and enables narrower acquisition windows. This, in turn, increases target capacity while minimizing the risk of missed peaks.

Together, the software and instrument enhancements bring unprecedented efficiency and precision to targeted proteomics. These capabilities streamline what has historically been one of the most labor-intensive aspects of targeted proteomics, giving researchers a workflow that is dramatically faster, more consistent, and inherently scalable. By merging intelligent method development and acquisition with engineered quantitative standards, the qRePS and Stellar mass spectrometer pairing becomes a highly efficient engine for delivering confident, high-quality plasma proteomics at scale.

"*What once took us two to three months of manual method development we completed in just two weeks. Stellar MS didn't just speed up the process; it fundamentally changed our expectations for how quickly high-quality targeted methods can be deployed.*"

Fredrik Edfors Arfwidsson
Assistant Professor, Royal Institute of Tecnology, Stockholm (KTH) and Co-Founder of ProteomEdge

Biology that drives clinically relevant research insights

Overall, this study revealed several meaningful and biologically intelligible patterns that offered clinically relevant research insights:

- Upregulated proteins: Inclusion of complement factors and inflammatory mediators, which showed progressive, trimester-associated increases
- Proteins that remained stable: Demonstrated by exceptionally low variance and highlighting the consistency of the workflow
- Downregulated proteins: Observation of specific metabolic regulators and carrier proteins that declined predictably over time

The ability to resolve these trends relied heavily on the absolute quantification strategy; the heavy-to-light ratios generated from co-digested qRePSs eliminated drift and preserved true biological signal. In effect, the workflow provided a quantitative anchor point that allowed subtle physiological changes to emerge with high confidence (Figure 5).

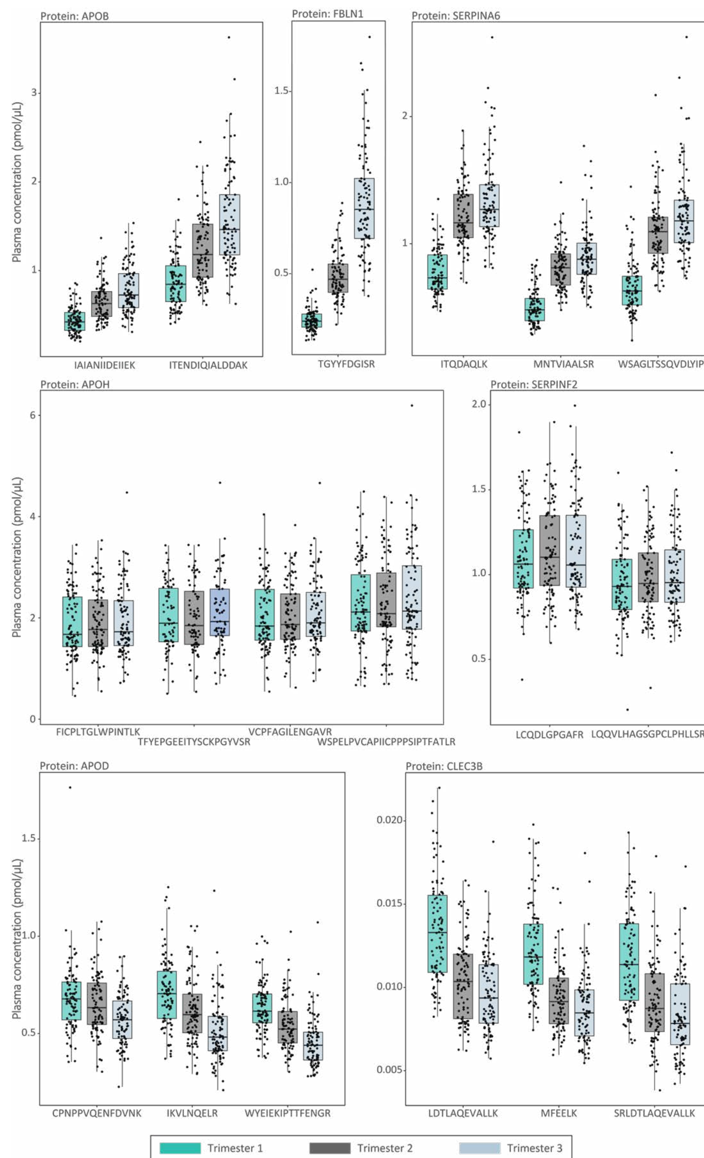

Figure 5. Protein abundance changes during pregnancy.
Multiple proteins showed characteristic abundance patterns across pregnancy. Examples include upregulated proteins (APOB, FBLN1, SERPINA6), proteins with relatively stable expression (APOH, SERPINF2), and downregulated proteins (APOD, CLEC3B). These profiles illustrate the ability of the workflow to detect physiologically relevant longitudinal changes.

This study not only validated qRePS in a demanding clinical research setting; it demonstrated the platform's suitability for large-scale, longitudinal, multi-plate MS workflows, where precision and reproducibility are essential for interpreting temporal biology. The data is available online at The Human Protein Atlas[1] with the aim of running up to 3,000 individuals covering all major diseases.

Thermo Fisher SCIENTIFIC

Conclusion

The integration of qRePS with high-throughput targeted MS on the Stellar mass spectrometer provides a practical framework for absolute quantification in plasma proteomics. By enabling co-digestion of standards and endogenous proteins, multi-peptide quantification, and process-controlled measurement, the workflow supports reproducible protein quantification across cohorts, timepoints, and large analytical batches.

When combined with Stellar MS capabilities, such as adaptive retention-time scheduling, fast MS/MS acquisition, and compatibility with automated method development, the approach enables efficient scaling from focused verification studies to larger longitudinal and translational research applications. The resulting data quality and quantitative consistency support confident biomarker evaluation while reducing manual intervention and workflow complexity.

Together, the qRePS-based standardization strategy and Stellar MS acquisition performance provide a robust foundation for plasma proteomics workflows requiring absolute quantification, reproducibility, and scalability across the biomarker development continuum.

Workflow impact

- **Consistent absolute quantification:** Enabled by co-digestion of recombinant standards and endogenous proteins
- **Higher confidence protein measurements:** Achieved through multi-peptide quantification per target
- **Reproducible results across studies**: Maintained across timepoints and longitudinal cohorts
- **Scalable, high-throughput analysis:** Supported by automated methods and adaptive RT on the Stellar mass spectrometer
- **Translationally relevant data:** Suitable for comparison across runs, plates, and studies

References

1. The Human Protein Atlas. *Proteins Absolutely Quantified by Targeted MS.* https://www.proteinatlas.org/humanproteome/blood/proteins+detected+by+targeted+ms (accessed February 18, 2026).

Acknowledgements

David Kotol[1], Fredrik Edfors[2], Mathias Uhlen[2], Henrik Everberg[1], and Stephanie Samra[3]

[1]ProteomEdge AB, Stockholm, Sweden

[2]Science for Life Laboratory, Department of Protein Science, CBH KTH Royal Institute of Technology, Stockholm, Sweden

[3]Thermo Fisher Scientific, San Jose, CA, USA

Thermo Scientific™ general lab equipment, consumables, and software are not intended for *in vitro* diagnostic purposes in accordance with our product documentation, manuals, and labels. They are designated for General Laboratory Use Only. Not for use in diagnostic procedures. These products have not been tested or validated for such applications and their use for *in vitro* diagnostic purposes may result in inaccurate results and, more seriously, health and safety risks. They are designated (designed, intended) for research, academic, and industrial purposes.

General Laboratory Equipment – Not For Diagnostic Procedures. © 2026 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Evosep and Evosep One are trademarks of Evosep ApS. ProteomEdge and DiscoveryEdge are trademarks of ProteomEdge AB. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. **CS004412-EN 0426**

thermo scientific