ATLAS ANTIBODIES

QPrEST

High Accuracy Targeted Proteomics

QPrEST

Stable Isotope-Labeled Protein Standard for Absolute Quantification using Mass Spectrometry

Within the field of proteomics, mass spectrometry (MS) is the method of choice for protein analysis[1]. Using MS, thousands of proteins from a trypsin digested complex sample can be identified in a single run. In addition to enabling protein identification, MS has also become a key tool for protein quantification. One strategy for accurate measurement of protein abundance is to spike in heavy isotope-labeled standards corresponding to the proteins of interest.

Figure 1. Schematic view over the workflow for protein quantification using QPrEST standards. The labeled QPrEST is spiked into the protein sample prior to trypsin digestion. The formed labeled and unlabeled peptides are then compared to determine the protein concentration.

Design and Workflow

The QPrESTs originate from the Human Protein Atlas project[2,3], where unlabeled (light) protein epitope signature tags (PrESTs) are used as antigens for antibody generation. QPrESTs are heavy isotope-labeled version of these proteins.

QPrESTs consists of 50-150 amino acids identical to a part of the corresponding human protein. The sequences are selected to have low similarity to other human proteins and cover regions with proteotypic peptides.

The QPrESTs are produced recombinantly in the presence of heavy isotope-labeled amino acids to generate protein standards suitable for MS-based quantification[4-6].

The QPrESTs are added to the sample prior to proteolytic digestion and the shared amino acid sequence of the QPrEST and the endogenous protein enables the QPrEST to correct for the variation introduced during the sample digestion (Figure 1).

The formation of multiple quantotypic peptides enables quantitative results from different peptides to validate each other.

The similar digestion efficiency of the QPrEST and the endogenous protein furthermore enables the use of miscleaved peptides, as they usually generate heavy to light ratios similar to the fully cleaved peptides.

Production and Quality Control

Heavy isotope-labeled QPrESTs are produced by expression in an Escherichia coli BL21(DE3) derivative, auxotrophic for lysine and arginine[7] followed by affinity purification using an N-terminal hexahistidine tag.

The cell cultivation is performed in a minimal autoinduction medium and isotopic incorporation is achieved by the addition of heavy isotope-labeled arginine and lysine (13C, 15N). The auxotrophy results in a near complete (>99%) isotopic incorporation as verified through the absence of peaks corresponding to unlabeled peptides in an MS spectrum (Figure 2).

Prior to storage, QPrESTs standards are lyophilized for maximal stability and to enable long-term storage. In order to avoid variation introduced during lyophilization step, the quality control is performed after lyophilization.

QPrEST purity (≥90%) is verified using the BioAnalyzer protein 230 purity assay and the correct protein molecular weight is confirmed using LC-MS analysis. For further confirmation of the protein identity, the presence of QPrEST peptides generated by tryptic digestion is verified using LC-MS/MS.

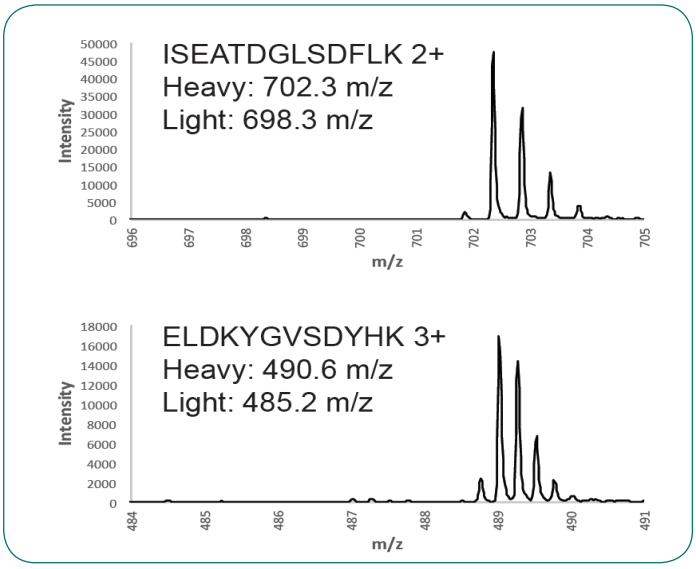

Figure 2. Verification of isotopic incorporation.
Analysis of QPrEST tryptic digests using ESI-MS shows that no peaks corresponding to unlabeled peptides can be detected.

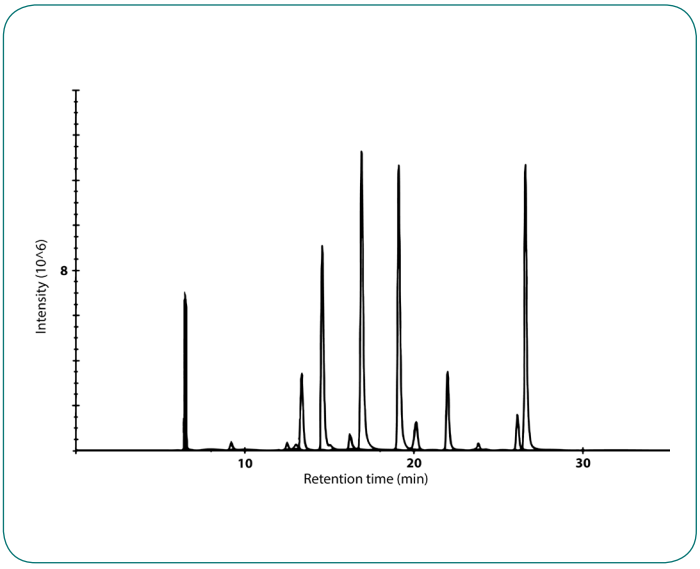

Figure 4.
Example chromatogram showing the distribution of QTag peptides over a 30 min LC gradient.

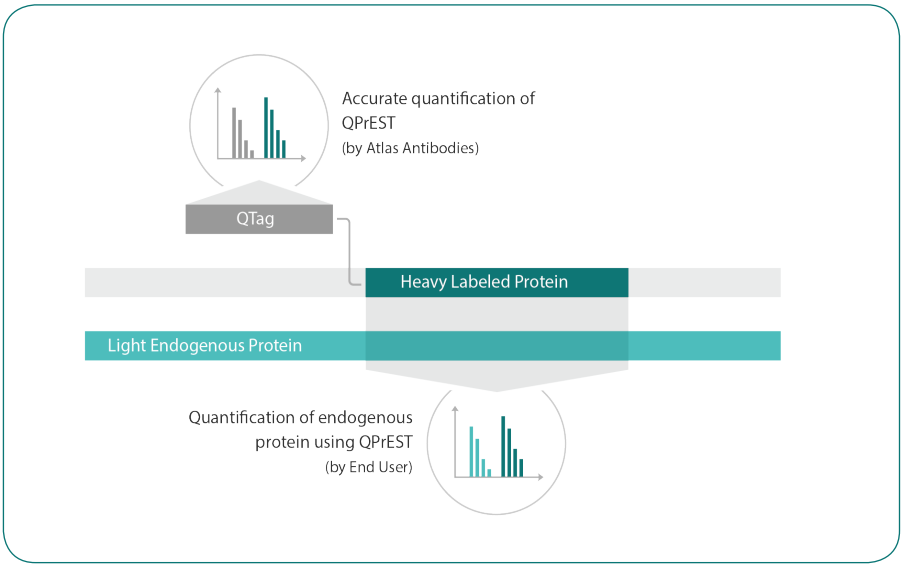

Figure 3. Schematic figure of a QPrEST standard.
The N-terminal part of the sequence consists of the QTag, used for purification and accurate quantification of the QPrEST using an unlabeled QTag. The C-terminal part of the sequence is identical to a portion of a human protein, this part is used for absolute quantification of the endogenous target protein.

Quantification

The N-terminal part of the QPrEST consists of a QTag sequence containing quantotypic peptides used for accurate quantification of the QPrEST (Figure 3).

An unlabeled, ultra-pure and amino acid analyzed QTag, is used as an internal reference in an LC-MS-QTOF setup where where ratios between the light and heavy QTag peptides are used to determine the absolute QPrEST concentration[5].

Accurate QPrEST concentration is determined based data generated at three separate occasions.

Furthermore, elution of QTag peptides is distributed over the LC gradient and therefore the peptides are also suitable to be used as retention time calibration standards (Figure 4).

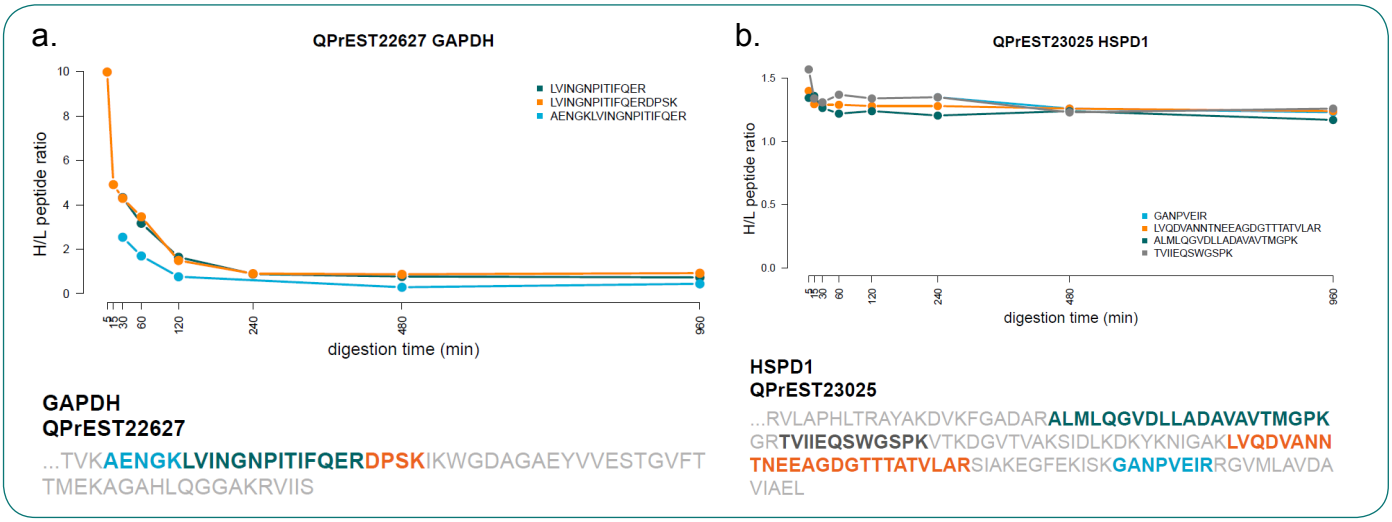

Figure 5.
Heavy to light (H/L) ratios for peptides originating from GAPDH (a) and HSPD1 (b) after different digestion times. QPrEST sequences are shown at the bottom and quantified peptides are highlighted in different colors.

Accuracy and Digestion

Digestion efficiency of QPrEST standards compared to endogenous proteins has been evaluated by comparing heavy to light peptide ratios generated after different digestion time points.

QPrEST standards were added to HeLa lysates directly after cell lysis and added in such amount that the generated heavy to light ratio was close to one. Digestion was stopped at eight different time points ranging from 5 min to 16 hours.

Samples were then analyzed on a Bruker Impact II mass spectrometer using data-dependent acquisition. Heavy to light ratios for at least two peptides from each QPrEST were determined and plotted against digestion time.

Figure 5 shows that peptide formation from QPrEST standards and endogenous protein reach equilibrium already after a few hours, thus enabling fast sample preparation. Data from different peptides targeting the same protein show comparable ratios after digestion equilibrium has been reached, this applies to both fully tryptic and miscleaved peptides.

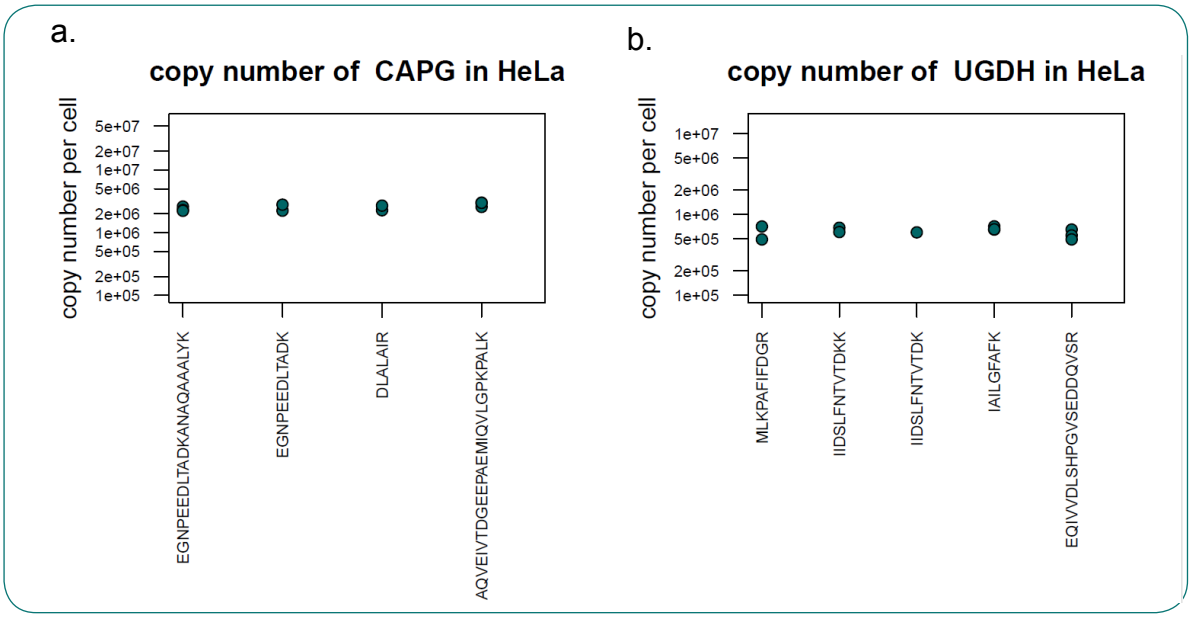

Figure 6.
Absolute quantification of two human proteins using QPrEST standards. The copy numbers of CAPG (a) and UGDH (b) were determined in HeLa cells.

Application Examples

Protein quantification in complex samples using QPrESTs can be performed either in singleplex or in a multiplex format where a set of QPrEST standards is added to the sample for parallel analysis of multiple target proteins[4,5].

Protein Quantification in Cell Lines

Figure 6 shows two examples of protein quantification where a set of QPrEST standards was spiked into a HeLa cell lysate directly after cell lysis and the sample was digested with trypsin using the filter-aided sample preparation (FASP) methodology[4].

After preparation, samples were analyzed on a Q Exactive HF mass spectrometer using a data-dependent acquisition setup.

The protein UGDH was quantified to 890,000 copies per cell using a total of five peptides generated from two separate QPrEST standards.

For CAPG, one QPrEST with a total of four peptides was used for quantification and the results show that this protein is present in HeLa at 2.5 million copies per cell.

Protein Quantification in Plasma

QPrEST standards have also been used for generation of targeted assays in human plasma samples, one example is shown in Figure 7.

Serial dilutions of a QPrEST targeting APOA4 were prepared and spiked into plasma samples to determine the range for which the assay gives a linear response. Samples were digested with trypsin using an in-solution sodium deoxycholate (SDC) protocol and then analyzed on a Q Exactive Plus mass spectrometer using a PRM targeted MS acquisition method.

The PRM data was processed using the Skyline software and extracted heavy to light ratios were plotted against the spiked-in QPrEST concentrations.

Figure 7a shows that, in total, both heavy and light peptides could be detected for four peptides at nine QPrEST spike-in levels and the response curve showed good linearity (R2 = 0.994) with a slope close to one across nearly 3 orders of magnitude for all included peptides. This data suggests that accurate APOA4 concentration can be determined also from a single spike-in if the ratio of the QPrEST and endogenous protein is between 1:20 and 20:1.

Figure 7b shows an example of where the APOA4 concentration in plasma was determined in triplicate using a QPrEST spike-in level close to the endogenous APOA4 level. All four peptides showed similar results and the concentration of APOA4 in this plasma sample was determined to 2.6 µM, based on median of the four peptides.

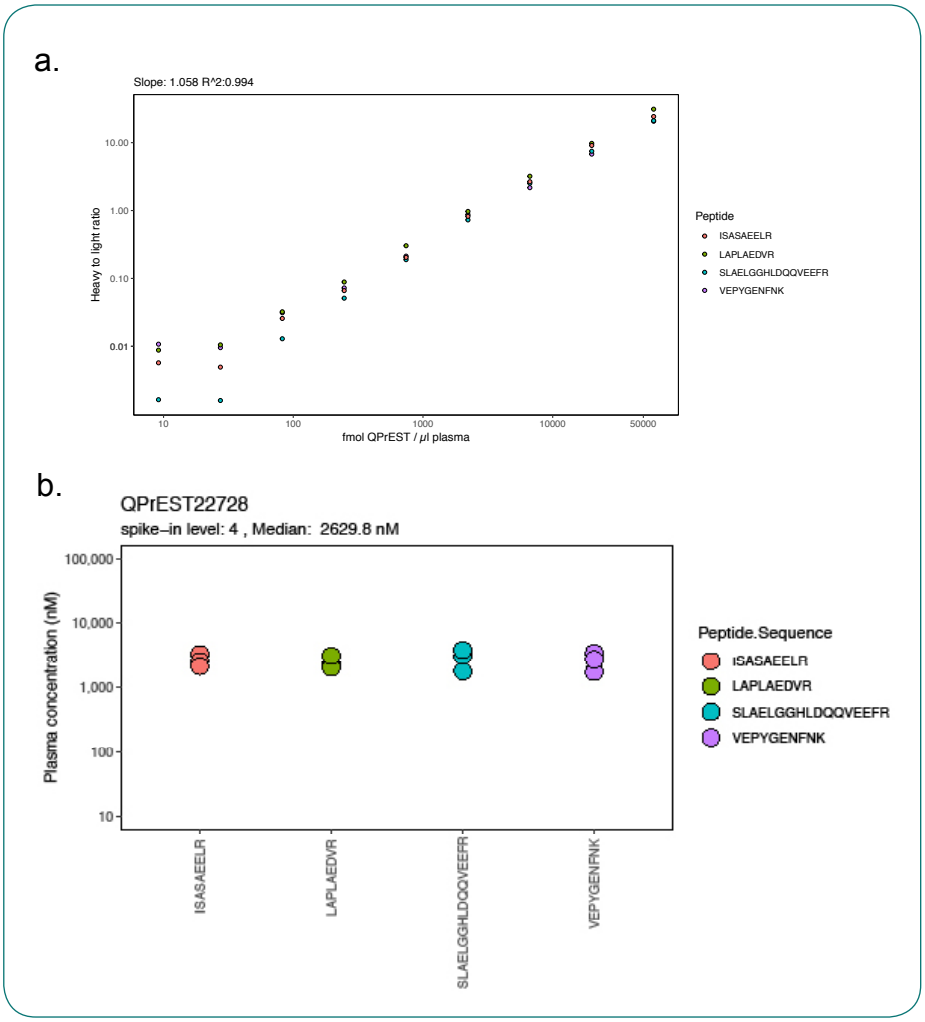

Figure 7.
Absolute concentrations of APOA4 in plasma samples were determined at different QPrEST spike-in levels (a). Absolute concentrations of APOA4 in plasma were determined using a spike-in level close to the endogenous APOA4 concentration (b).

QPrEST Standards in Published Research

The QPrEST-based protein quantification method was originally developed by Prof. Matthias Mann and Prof. Mathias Uhlén, who in recent years have used the approach in their respective laboratories, resulting in a number of publications.

In 2012, Zeiler et al.[5] showed how QPrEST standards (then referred to as SILAC PrESTs or heavy isotope–labeled PrESTs) can be used for multiplex protein quantification in HeLa cells. In their proof-of-principle study, absolute quantification of 40 human proteins was performed in parallel. The included proteins spanned a large concentration range, from FOS, with a cellular abundance of 6,000 copies, to vimentin with 20 million copies per cell.

In 2016, Edfors et al.[8] used a multiplex QPrEST mix to quantify 55 proteins across nine cell lines and eleven tissues in an effort to investigate the per gene correlation of protein and mRNA levels in the different samples. In this study they also used a QPrEST-based histone normalization strategy to allow for determination of copy numbers per cell also in tissues with varying cell densities.

Recently, a comparison of different MS standards as an alternative to stable-isotope labeled proteins (PSAQ) including QPrEST standards, heavy peptides and winged SIL (WiSIL) peptides, was performed by Oeckl et al.[9]. The included standards were used for quantification of alpha-synuclein in cerebrospinal fluid (CSF) using a selected reaction monitoring (SRM) MS setup. Comparisons regarding accuracy and precision showed that all approaches generated results with acceptable CV values (<15%), while the QPrESTs was the only standard yielding comparable concentration to the PSAQ strategy (deviation <15%).

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Summary

- QPrESTs are Stable Isotope-Labeled Protein Standards for Absolute Quantification using Mass Spectrometry
- QPrESTs are spiked in early and show a similar digestion efficiency to the endogenous proteins, thus increasing accuracy in quantification
- QPrESTs can be used to determine absolute protein amounts in multiple sample types, including cell lysate and plasma
- QPrESTs are pre-quantified, “Ready to Use” and available for the vast majority of the proteins in the human proteome