Isotope-labeled Multipeptide Standards for Quantitative Mass Spectrometry
QPrEST– Isotope-labeled multiprotein standards for quantitative mass spectrometry

Introduction
Within the field of proteomics, mass spectrometry (MS) has become the method of choice for protein analysis1. Using MS, thousands of proteins from a complex sample can be identified in a single run. Apart from enabling protein identification, MS has also become a key tool for protein quantification. One strategy to obtain highly accurate measures of protein abundance within a sample is to use a heavy isotope-labeled standard, preferably spiked into the sample at an early stage of the sample preparation procedure. In order to obtain reliable quantitative data, it is desirable to quantify multiple peptides for each target protein. Several types of isotope-labeled standards exist, one being Atlas Antibodies’ quantitative standard QPrEST. This standard possesses several beneficial properties such as high incorporation efficiency of heavy isotope-labeled amino acid residues. It also contains multiple unique tryptic peptides, increasing the reliability of the quantitative measurement of the corresponding endogenous target protein.

QPrEST isotope-labeled standards
The QPrEST standards are originally derived from the Human Protein Atlas project2,3, where unlabeled (light) recombinant protein fragments are used as antigens for antibody generation. These protein epitope signature tags (PrESTs) can be produced with incorporated heavy isotope-labeled amino acids to generate proteins with unique properties that make them suitable as internal standards for mass spectrometry-based quantification4-6. All QPrESTs contain a stretch of 50-150 amino acids identical to a target human protein sequence including at least two unique tryptic peptides. The QPrEST standard can be added to the sample at an early stage of the sample preparation workflow, decreasing the variation introduced during for example proteolytic cleavage. An N-terminal quantification tag (QTag) is present in all QPrEST standards, and is used for accurate quantification of the QPrEST using an unlabeled QTag protein as internal reference (Fig 1). The QTag consists of a hexahistidine tag genetically fused to an albumin binding protein (ABP) sequence derived from streptococcal protein G. Quantification of QPrEST standards is based on quantitative analysis of multiple tryptic peptides spanning the QTag sequence.

Proteome coverage
The QPrEST product catalog contains over 20,000 products, of which over 70% have at least one experimentally verified proteotypic peptide, based on data from PeptideAtlas7 (Fig 2). In total, the currently available QPrEST standards target more than 13,000 human proteins, of which more than 40% are covered by multiple (up to five) QPrEST standards.

Production and quality control
Heavy isotope-labeled QPrESTs are expressed in an Escherichia coli BL21(DE3) derivative, auxotrophic for lysine and arginine8. The cell culture is performed in a minimal autoinduction medium and isotopic incorporation is achieved by the addition of heavy isotope-labeled arginine and lysine (15C, 15N) to the culture. The QTag part of the protein sequence contains an N-terminal hexahistidine tag used for affinity purification of the QPrEST standard. After purification, QPrEST purity is verified using SDS-PAGE and the correct protein molecular weight is confirmed using ESI-MS analysis (Fig 3).

The auxotrophic E. coli strain contains deletions in the lysA and argA genes making the cells unable to survive without the addition of arginine and lysine to the growth medium (Fig 4A). 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 (Fig 4B).

Figure 1. Schematic figure of a QPrEST standard (green). The N-terminal part of the sequence consists of the QTag sequence, used for purification and accurate quantification of the QPrEST using an unlabeled QTag (orange). The C-terminal part of the sequence is identical to a portion of a human protein (blue). This part is used for absolute quantification of the endogenous target protein.

Figure 2. Distribution of QPrEST products based on number of unique tryptic peptides (blue bars). The number of QPrESTs within each group with at least one tryptic peptide present in PeptideAtlas is also presented (orange bars).

Figure 3. Quality control of QPrEST standard. (A) SDS-PAGE is used to determine protein purity and (B) the protein molecular weight is verified with ESI-MS.
Accurate QPrEST quantification
Labeled QPrEST standards are accurately quantified using the QTag part of the protein. An unlabeled version of the QTag is used as internal reference in an MS-based setup where multiple QTag-derived peptides are used to quantify the QPrEST standard\(^5\). A high purity of the QTag is ensured through multiple affinity purification steps based on both N- and C-terminal purification handles and the QTag concentration is accurately quantified using amino acid analysis. The heavy isotope-labeled QPrEST is mixed with the unlabeled QTag protein and the sample is digested. Peptides are further analyzed using an LC-ESI-QTOF setup where ratios between light and heavy QTag-derived peptides are used to determine the absolute QPrEST concentration (Fig 5). The analysis is not limited to fully cleaved tryptic peptides, as the equal digestion efficiency between QPrEST and QTag results in very similar peptide ratios for fully cleaved tryptic peptides and peptides containing one or two intact tryptic cleavage sites. Accurate quantification of the endogenous target protein depends on the quantitative precision of the added standard. Based on data from three replicate experiments, the concentration is determined with an imprecision below 10%.

The QPrEST standards are stable and no effect on the QPrEST concentration has been observed after repeated freeze-thaw cycles.

Figure 4.
Verification of isotopic incorporation. (A) The Lys and Arg auxotrophic E. coli BL21(DE3) strain is dependent on addition of Arg and Lys when grown on minimal medium. (B) Analysis of QPrEST tryptic digests using ESI-MS shows that no peaks corresponding to unlabeled peptides can be detected.

Figure 5.
QPrEST quantification using three example QTag peptides. Extracted ion chromatograms are shown to the left. Extracted spectra with peaks corresponding to both labeled and unlabeled peptides are shown to the right. The determined L/H ratio for each peptide is also presented. The three peptides are marked in the QTag sequence at the bottom of the figure.
**Application example of QPrESTs as internal standards**

Protein quantification using QPrEST standards can be performed either in single-plex, or in a multi-plex format where a set of QPrEST standards is added to the sample for parallel analysis of multiple target proteins. QPrEST standards have been used as internal references to determine the copy number of proteins in cell lines\(^4,5\). A set of QPrEST standards was spiked into a HeLa cell lysate directly after cell lysis and the sample was digested using the filter-aided sample preparation (FASP) methodology. Generated peptides were further fractionated into six samples using strong anion exchange chromatography in a pipette tip format. Peptide fractions were desalted and separated using a 3 h LC gradient prior to injection on a QExactive mass spectrometer. Fig. 6 shows two examples of protein quantification using QPrEST standards\(^6\). The protein UGDH was quantified using a total of five peptides generated from two separate QPrEST standards, resulting in a determined copy number of 890,000 copies per cell. CAPG was quantified using one QPrEST and a total of four peptides. The copy number was for this protein determined to be 2.5 million copies per cell.

![copy number of CAPG in HeLa](image)

**Figure 6.** Absolute quantification of two human proteins using QPrEST standards. The copy numbers of CAPG and UGDH were determined in HeLa cells. Quantiﬁed peptides are shown on the x axis and determined copy numbers on the y axis. Each circle represents data from one of three replicate analyses.

**Summary**

- QPrESTs are recombinantly produced, heavy isotope-labeled, multipeptide standards for MS-based absolute quantification
- The product catalog contains over 20,000 bioinformatically selected QPrEST standards with proteome-wide coverage
- An accurate MS-based setup with an unlabeled internal QTag reference standard is used for precise QPrEST quantification to ensure reliable downstream results
- QPrEST standards have been used to determine absolute protein copy numbers in different cell lines

REFERENCES


atlasantibodies.com

Our website provides you with easy access to all characterization data, and online ordering via our web shop. You can also send your order to order@atlasantibodies.com.

Or send an e-mail to support@atlasantibodies.com to discuss any matters regarding protein quantification. *You’ll find we’re Totally Human.*