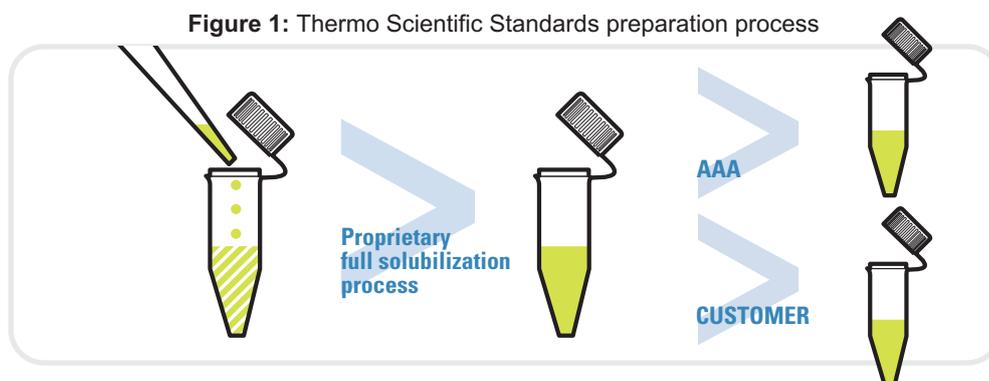


# MILESTONES towards PROTEOMIC STANDARDS

## Thermo Scientific HeavyPeptides™ AQUA

### Protein Quantitation by Mass Spectrometry

One of the key challenges in Proteomics is the quantitation of proteins at very low concentrations in complex protein mixtures. Assays that may exist lack absolute specificity and are difficult to multiplex. This is particularly true for disease biomarkers used for diagnostics, treatment development and monitoring<sup>1</sup>.



Quantitative mass spectrometry for small molecules<sup>2</sup> is based on the well established method of isotopic dilution. Due to its absolute specificity, sensitivity<sup>3</sup> and high multiplexing potential<sup>4</sup> this technique is quickly adopted for peptide quantitation<sup>5,6,7</sup> and absolute protein quantitation<sup>8,9</sup> in complex matrices.

To achieve absolute quantitative proteomics, proteins are digested with a protease like trypsin and proteotypic<sup>10</sup> peptides are used as stoichiometric surrogate. Accurate absolute quantitation is achieved by spiking the sample with isotopic labeled standards, also known as HeavyPeptides.

#### STEP 01:

##### Proteotypic peptide selection

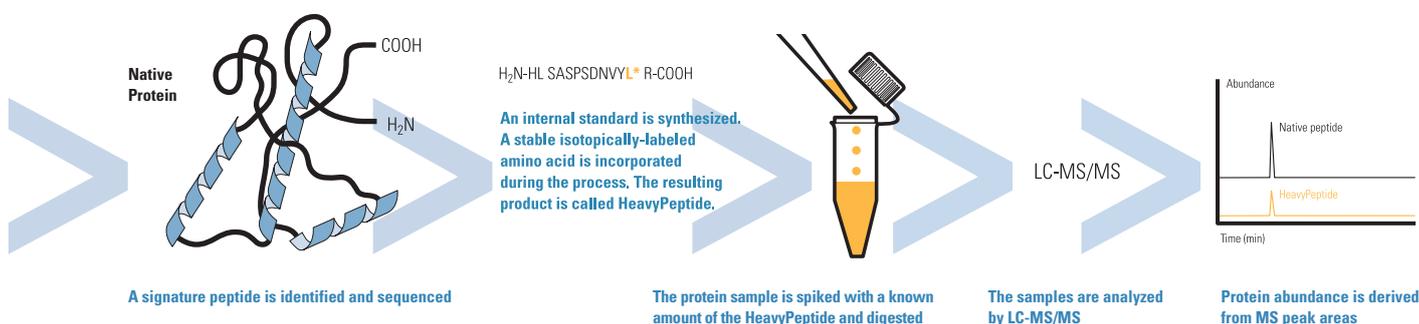
Starting with a software assisted decision<sup>11</sup> e.g. Thermo Scientific PinPoint ([www.thermoscientific.com/pinpoint](http://www.thermoscientific.com/pinpoint)), it is common to get multiple proteotypic peptide candidates per protein. Software selected peptides are tested on the mass spectrometer equipment to establish SRM/MRM protocols.

#### STEP 02:

##### Quantitation

Protease digested samples are spiked with known quantities of synthetic stable-isotope labeled peptides – HeavyPeptides – as internal standards. Multiplexing potential is very high and recent equipment and software developments are further increasing that unique ability.

**Figure 2: HeavyPeptides Workflow**



## HeavyPeptides grades:

Within our portfolio you will find the HeavyPeptide grade meeting your precision and budget requirements for absolute and relative quantitation.

### AQUA Ultimate

HeavyPeptides AQUA Ultimate are provided fully solubilized with a concentration precision equal or better than  $\pm 5\%$ . Best choice for biomarker validation and for experiments demanding ultimate quantitative precision and reproducibility from batch to batch.

### AQUA QuantPro

HeavyPeptides AQUA QuantPro are provided fully solubilized with a concentration precision equal or better than  $\pm 25\%$ . Ideal solution for biomarker verification.

### AQUA Basic

HeavyPeptide AQUA Basic are provided lyophilized and are more adequate for relative quantitation. The batch to batch consistency is difficult to predict.

### Crude

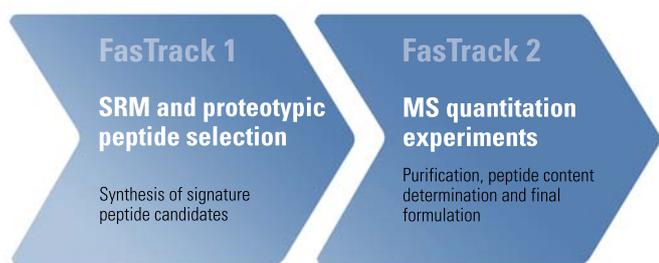
HeavyPeptides Crude are provided non purified in 96 well plate format. They are designed to assist the proteotypic peptide selection during SRM/MRM setup.

## HeavyPeptides – the ideal tool for post-translational modification quantitation.

HeavyPeptides can be prepared with almost all covalent modifications (e.g. phosphorylation, methylation, acetylation, etc). They are chemically identical to naturally occurring post-translational modifications. With their absolute specificity, HeavyPeptides represent the most powerful tool for relative and absolute quantitation of post-translational modified proteins.

### Applications

- Biomarker discovery, verification, validation
- Functional quantitative proteomics<sup>1,12</sup>
- RNAi result confirmation
- Pharmacokinetics
- Metabolomics
- Clinical biochemistry for drug and metabolite monitoring
- Anti-doping testing
- Protein expression monitoring
- Pathway validation
- Cell signaling profiling
- Allergen quantitation
- Systems biology Pharmacoproteomics<sup>13,14</sup>



### Assay development booster: FasTrack™ Service

The FasTrack service is designed for accelerating assay development within a controlled budget environment. FasTrack service is available for both HeavyPeptides AQUA Ultimate and QuantPro grade and offers a 2-step-approach:

#### Step 1 – FasTrack 1

Crude HeavyPeptide is synthesized within 8 days. 100ug are shipped for proteotypic peptide selection and assay development. The rest of the peptide is kept in stock for the optional FasTrack 2.

#### Step 2 – FasTrack 2

The crude peptide from FasTrack 1 is purified to reach a minimum purity of 97%, followed by full solubilization and concentration measurement.

FasTrack 2 is optional.

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