Quantification of the Short Lived Yeast Transcription Factor RPN4 Using Targeted Proteomics and SpikeTides™

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Introduction
Quantitative targeted proteomics has become a widely used technique in biology. Shotgun proteomics experiments enable the identification of numerous proteins in biological samples. However, the dynamic range of this technique is limited. Low abundance proteins are rarely observed in such experiments. With targeted proteomics the dynamic range can be extended considerably. Synthetic peptides are used as standards to determine the relative or absolute quantity of proteotypic peptides obtained from tryptic digestion of protein-containing biological samples. SpikeTides™ are small-scale, inexpensive, heavily labeled or non-labeled and/or absolutely quantified peptides for SRM and MRM assays.¹

Variants of SpikeTides™
SpikeTides™ address all peptide needs of targeted MS-based proteomics (Tab. 1).

<table>
<thead>
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<th>Development of SRM assays</th>
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<tr>
<td>SpikeTides Small scale, unpurified proteotypic peptides (&gt;50nmol)</td>
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<tr>
<td>Relative Quantification</td>
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<tr>
<td>SpikeTides_L SpikeTides™ with heavily labeled C-terminal lysine or arginine (Arg M + 10 or Lys M + 6)</td>
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<tr>
<td>Absolute Quantification</td>
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<tr>
<td>SpikeTides_TQ/SpikeTides_TQL SpikeTides™ with unlabeled (TQ) or heavily labeled (TQL) C-terminal lysine or arginine and absolutely quantified using a proprietary Quantitag. Aliquots of 5 x 1 nmol target peptide are delivered.</td>
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Synthesis & Quantification
SpikeTides™ are usually prepared via SPOT synthesis², which is the high-throughput synthesis of peptides on cellulose membranes. After synthesis, the peptides are cleaved off the membrane and transferred into 96- or 384-well plates. At JPT, this procedure yields up to 50,000 individual peptides per week. SpikeTides™ can be rapidly and inexpensively quantified using a unique quantification tag (Fig. 1). The tag is proteolytically labile and has UV-absorption properties that differ from those of the peptide, allowing quantification via HPLC in comparison to a standard.

Fig. 1: Concept of SpikeTide™ quantification.

The quantification tag is designed to be very hydrophilic and easily separated from the proteotypic peptides after digestion in standard SRM/MRM setups. It also becomes apparent that tag cleavage can be efficiently accomplished in mixtures of several SpikeTides. This is exemplified in Fig. 3. SpikeTides are suitable for the efficient establishment of MRM assays⁴,⁵. Isotopically labelled SpikeTides are used for the relative (SpikeTides_L) or absolute (SpikeTides_TQL) quantification of proteins in biological samples⁶.

Fig. 2: Tag cleavage efficiency for 40 SpikeTides™_TQL [FLDALHQVF-X-Y-Tag, by LC-UV at 350nm].

Fig. 3: Outcome of a typical trypsin-mediated tag cleavage experiment for a mixture of 20 SpikeTides. Efficient cleavage of the tag is observed.

Proof of Concept
Targeted proteomics with SpikeTides™ was used for the identification and quantification of a short lived transcription factor of Saccharomyces cerevisiae Rpn4⁷,⁸. This is a central protein in the negative feedback circuit of proteasome homeostasis. Rpn4 has a quick turnover and is found in low concentrations under normal growth conditions. The transcription of RPN4 is strongly up-regulated under stress conditions like the addition of heavy metal ions.

Fig. 4: Quantification of the yeast transcription factor Rpn4 using SpikeTide_TQL in targeted proteomics. wt and rpn4 cells were treated with CdCl₂ to induce Rpn4 production. Tryptic digests of total cell extracts were analyzed on a Q-TRAP-4000 mass spectrometer in MRM mode using an ESI setup.

Conclusion
SpikeTides™ are cost-effective peptides that allow high-speed SRM assay development and protein quantification with almost unlimited coverage through entire proteomes. They use a new approach to absolutely quantify peptides and enable the monitoring of cellular regulation by incorporation of post-translational modifications.

References

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