

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

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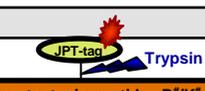
Introduction

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).

Tab. 1: Variants of SpikeTides™.

Development of SRM assays	
SpikeTides Small scale, unpurified proteotypic peptides (>50nmol)	proteotypic peptide R/K
Relative Quantification	
SpikeTides_L SpikeTides™ with heavily labeled C-terminal lysine or arginine (Arg M + 10 or Lys M + 8)	proteotypic peptide R*/K*
Absolute Quantification	
SpikeTides_TQ/SpikeTides_TQL SpikeTides™ with unlabeled (TQ) or heavily labeled (TQL) C-terminal lysine or arginine and absolutely quantified using a proprietary Quanti-Tag. Aliquots of 5 x 1 nmol target peptide are delivered.	 proteotypic peptide R*/K*
	* residue uniformly ¹³ C and ¹⁵ N labeled * residue optionally uniformly ¹³ C and ¹⁵ N labeled

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.

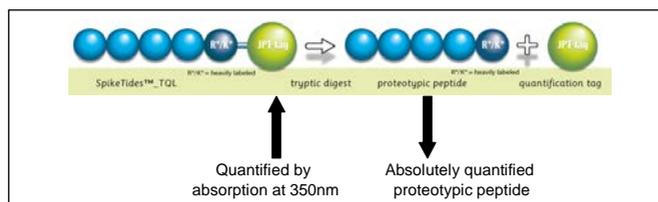


Fig. 1: Concept of SpikeTide™ quantification.

Fig. 2 shows that the cleavage efficiency for removing the tag from 40 SpikeTides™_TQL was ≥96 % in all cases, with the exception of only one peptide which bears a DK bond (coloured orange). As it is known that bonds between D and K are often cleaved slowly by trypsin³, peptides with DK-unit are not recommended as proteotypic peptides for SRM assays.

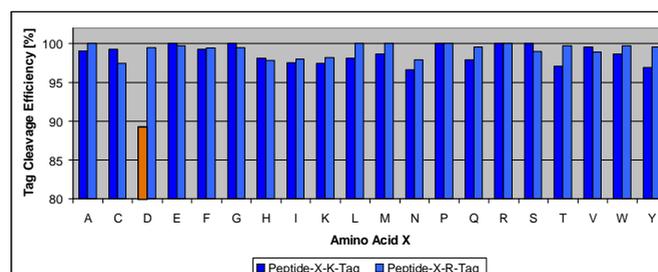


Fig. 2: Tag cleavage efficiency for 40 SpikeTides™_TQL [FLDALHQVF-X-Y-Tag, by LC-UV at 350nm].

The quantification tag is designed to be very hydrophilic and easily separated from the proteotypic peptides after digestion in standard SRM/MRM setups. Also it 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 assays^{4,5}. Isotopically labelled Spiketides are used for the relative (SpikeTides_L) or absolute (SpikeTides_TQL) quantification of proteins in biological samples⁶.

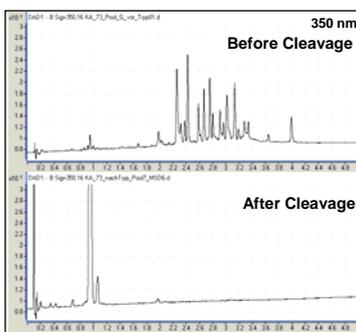


Fig. 3: Outcome of a typical trypsin-mediated tag cleavage experiment for a mixture of 20 SpikeTides. Efficient cleavage of the tag is observed.

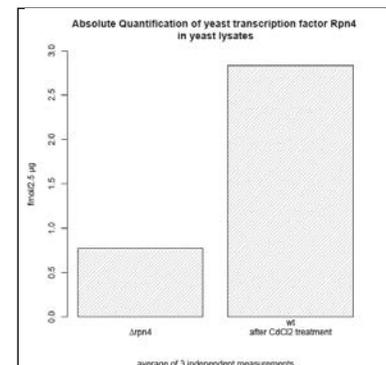


Fig. 4: Quantification of the yeast transcription factor Rpn4 using SpikeTide_TQL in targeted proteomics. wt and *rpn4* cells were treated with CdCl₂ to induce Rpn4 production. Tryptic digests of total cell extracts were analyzed on a Q-TRAP-4000 mass spectrometer in MRM mode using an ESI setup.

Proof of Concept

Targeted proteomics with SpikeTides™ was used for the identification and quantification of a short lived transcription factor of *Saccharomyces cerevisiae* Rpn4^{7,8}. 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.

Tryptic digests of lysates of a wild-type and *rpn4* deletion strain were spiked with SpikeTides_TQL. The detected amount for the wt-strain was as few as 8 copies per cell (Fig. 4).

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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