

SpikeTides™ – Small Scale & Low Cost Peptide Standards for Large Scale Proteomics

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Introduction

Targeted proteomics is dependent on custom peptides as standards for assay development and protein quantification. However, due to laborious synthesis and quantification procedures, currently available peptides have a high price of several hundred €/USD per peptide. 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	

SpikeTide™ Synthesis

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.

SpikeTide™ Quantification

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.

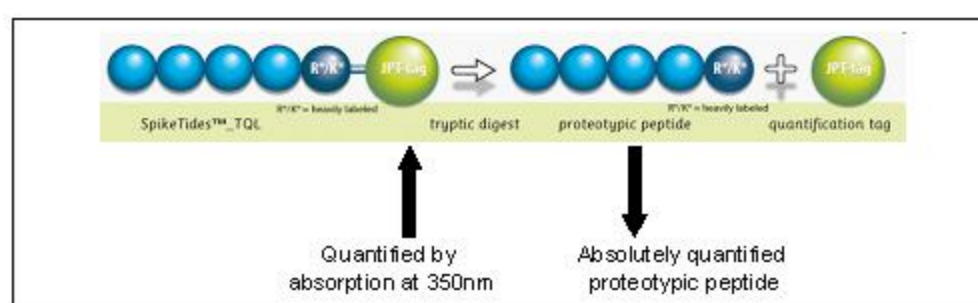


Fig. 1: Concept of SpikeTide™ quantification.

In Fig. 2 calibration curves for five tagged peptides and the standard are shown. The quantification is linear and independent of the peptide sequence. Overall error (SD) for quantification is 5.4%.

Fig. 3 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.

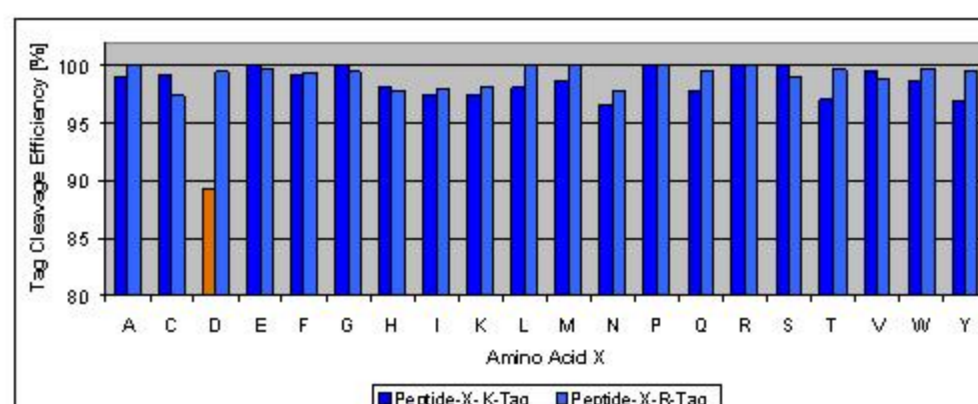


Fig. 3: Tag cleavage efficiency for 40 SpikeTides™_TQL [FLDALHQVF-X-Y-Tag, by LC-UV at 350nm].

The quantification tag is designed to be very hydrophilic to be easily separated from the proteotypic peptides after cleavage in standard SRM/MRM setups. This is exemplified in Fig. 4.

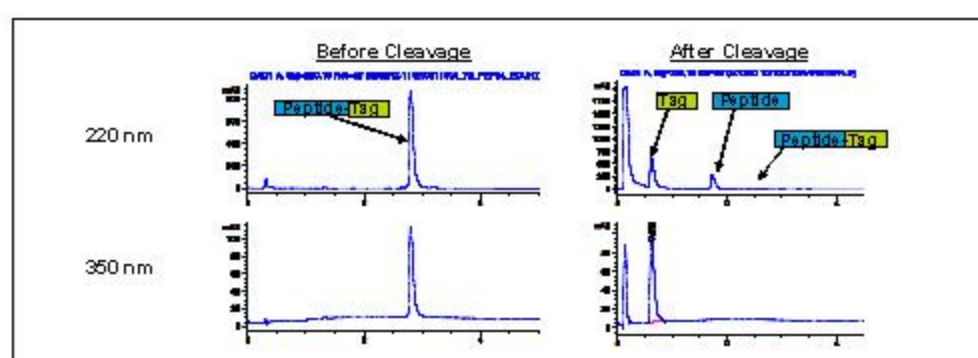


Fig. 4: Outcome of a typical tag cleavage experiment (Peptide = SPEVLLGSAR).

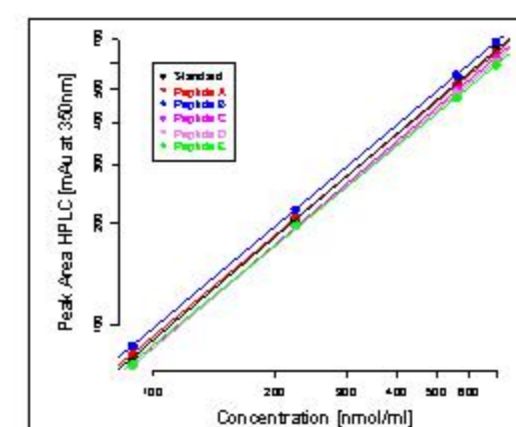


Fig. 2: Straight calibration lines for the quantification of five different SpikeTides™_TQL.

Applications

SpikeTides™ were used for SRM assay establishment in *Saccharomyces cerevisiae*, with a detection limit of less than 50 protein copies per cell,⁴ and for proteome-wide detection of all kinases and phosphatases in yeast.⁵ Recently, SpikeTides™ were used to validate MRM assays to detect biomarkers in the feces of patients with colorectal cancer.⁶ SpikeTides™ are also successfully applied in a large scale project aiming at a complete map of the human proteome by MS.⁷

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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