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Innovative Peptide Solutions

# SpikeTides™—proteotypic peptides for large-scale MS-based proteomics

Targeted proteomics, as an efficient and sensitive technology for protein identification and quantification, is dependent on the availability of custom peptides for assay development and absolute quantification. For absolute protein quantification the peptides must be heavily labeled and quantified. Current workflows rely on peptides that are prepared by labor-intensive resin-based peptide synthesis. The result is a high price per peptide, thus prohibiting large-scale projects.



SpikeTides™ are an innovative way to overcome these limitations. They are small-scale, inexpensive, heavily labeled or nonlabeled and/or absolutely quantified peptides for single reaction monitoring (SRM) and multiple reaction monitoring (MRM).

Targeted mass spectrometry (MS)–based proteomics such as SRM and MRM are used for the detection and quantification of proteins in complex samples such as biological fluids or cell lysates[1]. The accessible dynamic range of protein concentrations with detection limits as low as 50 copies of a protein per cell[2] led to the broader acceptance of the approach.

Proteotypic peptides are generated by protease-mediated digest of a protein. In SRM and MRM assays, distinctive proteotypic peptides from a protein are used for the identification of specific precursor- and fragment-ion masses in a mass spectrometer. Based on this, combined chromatographic and MS assays for single peptides and—by inference—for proteins are established. The optimized parameters can be used for the routine identification and quantification of the corresponding protein.

A growing number of SRM parameters are available in databases (such as www.srmatlas.org). However, the availability of peptides for assay development and quantification is still a bottleneck. High-throughput SPOT synthesis is a cost-effective way for the production of huge numbers of peptides. With this method, thousands of proteotypic peptides and their heavily labeled counterparts can be produced within days at a fraction of the cost of classical synthesis. To drive absolute peptide quantification to higher throughput and cost effectiveness, we have developed a quantification method that is based on a proteolytically cleavable quantification tag with unique spectrophotometric properties.

## Peptide synthesis

SpikeTides™ are usually prepared via SPOT synthesis[3], which is the Fmoc-based high-throughput solid-phase synthesis of peptides on cellulose membranes. Post-translational modifications, incorporation of heavily labeled amino acids and conjugations are possible. After synthesis, the peptides are cleaved off the membrane, transferred into ready-to-use 96- or 384-well plates and freeze dried. At JPT, this procedure yields up to 50,000 individual peptides per week.

## Peptide quantification

SpikeTides™ are rapidly and inexpensively quantified using a unique quantification tag. 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. **Figure 1** shows calibration curves for tagged peptides and the standard. The quantification is linear over the tested concentration range (100–700 nmol $ml^{-1}$) and independent of peptide sequence. Overall error (SD) for quantification is 5.4%.

## Establishment of SRM or MRM assays

Initially, putative proteotypic peptide sequences from proteins of interest are selected (for instance, from PeptideAtlas (www.peptideatlas.org) or PRIDE (www.ebi.ac.uk/pride/)), or predicted. Candidate peptides are synthesized and injected into an HPLC system coupled to a triple quadrupole mass spectrometer. Predominant peptide fragments are chosen for each peptide by selection of the peptide ion, fragmentation and selection of an indicative fragment ion. Subsequently, the MS parameters can be optimized to maximize the sensitivity of the assay. After validation, characteristic assay parameters are sufficient to repeat the assay. Consequently they have to be established only once for a specific type of mass spectrometer and fragmentation method. Assay development can be carried out in a multiplexed fashion using over 100 peptides in a single run. For a comprehensive description, see Picotti *et al.*[4]

## Applications

JPT has developed a product class, SpikeTides™, that addresses all peptide needs of targeted MS-based proteomics (**Table 1**).

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# APPLICATION NOTES

**Table 1** | Variants of SpikeTides™

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|---|---|
| **Development of SRM or MRM assays** | |
| **SpikeTides™**<br>Small-scale, unpurified proteotypic peptides (>50 nmol) | Proteotypic peptide ⋮ R/K |
| **Relative quantification** | |
| **SpikeTides_L**<br>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**<br>SpikeTides™ with unlabeled (TQ) or heavily labeled (TQL) C-terminal lysine or arginine and absolutely quantified using a proprietary Quanti-Tag. Proteotypic peptides are released from tag by tryptic digestion. Aliquots of 5 ×1 nmol target peptide are delivered. | JPT-tag, Trypsin<br>Proteotypic peptide ⋮ R#/K# |

* Residue uniformly $^{13}$C- and $^{15}$N-labeled
\# Residue optionally uniformly $^{13}$C- and $^{15}$N-labeled

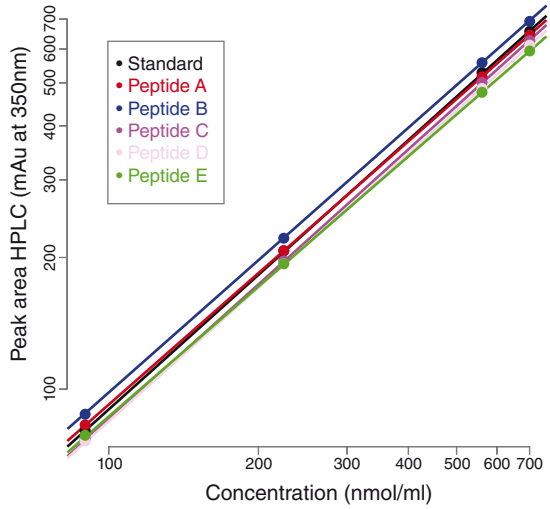


**Figure 1** | Straight calibration lines for the quantification of five different SpikeTides_TQL vs. corresponding peptides quantified by amino acid analysis.

**SpikeTides™ for development of SRM or MRM assays:** Synthetic proteotypic peptides for SRM/MRM assay development are unlabeled and not quantified.

**SpikeTides_L for relative protein quantification:** Uniformly $^{13}$C- and $^{15}$N-labeled arginine or lysine is used at the C terminus of SpikeTides_L. These amino acids were selected because all proteotypic peptides that result from tryptic digestion of proteins contain C-terminal arginine or lysine. After the SpikeTides_L are spiked into the sample, two sets of fragment ions can be detected: heavily-isotope labeled (from SpikeTide_L, mass difference +8 Lys, +10 Arg) and non-labeled (from digested protein). Spiking in SpikeTides_L into different biological samples permits the relative quantification of proteins from sample to sample.

**SpikeTides_TQ/SpikeTides_TQL™ for absolute protein quantification:** Unlabeled or heavily-isotope labeled peptides are quantified using a covalently bound UV-active quantification tag that is cleaved by tryptic digests in standard SRM or MRM workflows. The defined concentration of the SpikeTide_TQ or SpikeTide_TQL allows the absolute quantification of a protein in a biological sample. The tag is designed to be of low molecular weight and hydrophilic, minimizing interference in HPLC separation and MS detection.

## Examples of uses

SpikeTides™ are ideal tools for the establishment of assays to measure the abundance of many proteins in a wide variety of biological samples. This has been demonstrated in *Saccharomyces cerevisiae*, in which the detection limit for low-abundancy proteins by SRM was less than 50 protein copies per cell[2]. Furthermore, the development of SRM assays for proteome-wide detection of all kinases and phosphatases in yeast using SpikeTides™ has been described[4]. SpikeTides™ are successfully applied in a large scale project aiming at a complete map of the human proteome by MS, initiated by the Institute for Systems Biology (Seattle) and the Swiss Federal Institute of Technology (Zürich).

Global and targeted approaches for biomarker discovery, as well as deciphering biological pathways with a medical focus, benefit from SpikeTides™. Recently, SpikeTides™ were used to validate MRM assays to detect biomarkers in the feces of patients with colorectal cancer[5]. Hewel *et al.* set up a flexible, rapid and cost-effective assay system for the detection and quantification of low-abundance components in signaling pathways of human embryonic stem-cell populations[6]. Here, the SpikeTides™ concept was used for the first combined absolute quantification of key nodes in a regulatory pathway by MS-based proteomics.

## Conclusion

SpikeTides™ are cost-effective peptides that allow the full utilization of MS-based proteomics by opening new dimensions in sensitivity and almost unlimited coverage through entire proteomes. They provide access to high-speed assay development and protein quantification and use a new approach to absolutely quantify peptides, thus eliminating laborious and expensive peptide purification and amino acid analysis. Additionally, the flexibility of chemical synthesis of SpikeTides™ permits monitoring of cellular regulation by incorporation of post-translational modifications.

## Link to the supplier's website

www.jpt.com


1. Doerr, A. Targeted Proteomics. *Nat. Methods* **7**, 34 (2010).
2. Picotti, P., Bodenmiller, B., Mueller, L.N., Domon, B., & Aebersold, R. Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics. *Cell* **138**, 795–806 (2009).
3. Wenschuh, H. *et al.* Coherent membrane supports for parallel microsynthesis and screening of bioactive peptides. *Biopolymers* **55**, 188–206 (2000).
4. Picotti, P. *et al.* High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nat. Methods* **7**, 43–46 (2010).
5. Ang, C.S. & Nice, E.C. Targeted in-gel MRM: a hypothesis driven approach for colorectal cancer biomarker discovery in human feces. *J. Proteome Res.* **9**, 4346–4355 (2010).
6. Hewel, J.A. *et al.* Synthetic peptide arrays for pathway-level protein monitoring by liquid chromatography-tandem mass spectrometry. *Mol. Cell. Proteomics* **9**, 2460–2473 (2010).